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A LABORATORY MANUAL
OF
ORGANIC CHEMISTRY
FOR
MEDICAL STUDENTS

BY

MATTHEW STEEL, PH.D.

PROFESSOR OF ORGANIC AND BIOLOGICAL CHEMISTRY, THE LONG ISLAND
COLLEGE HOSPITAL, BROOKLYN, NEW YORK.

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PREFACE

THIS manual was originally compiled as a laboratory guide in organic chemistry for the medical students of the Long Island College Hospital, Brooklyn, New York.

While writing these notes, the author kept one object constantly in view, namely, the selection of experiments that would be of real value to medical students.

The recent development of biological chemistry has created a demand for a much broader training in experimental organic chemistry than was formerly required of medical students. In this manual an attempt has been made to fulfill this need without burdening the student with a mass of unessential data.

In nearly every experiment definite quantities of chemicals have been specified, the object being twofold: to prevent the unnecessary waste of material, and to insure the success of the experiment.

Each student is expected to record his own observations, and for this reason alternate pages of the manual have been left blank.

To induce the student to correlate the facts observed in the laboratory with the theoretical matter taught in the lecture room and in the text-books, the experiments are accompanied with questions which the student is supposed to answer.

Much of the data contained in the chapter on alkaloids was obtained from Autenrieth's book "Detection of Poisons and Powerful Drugs." Authorized translation by W. H. Warren, Ph.D. (Blakiston). The author wishes to express his gratitude to Messrs. Blakiston &

Company for the courtesy shown in permitting the use of these data.

The author also wishes to acknowledge his indebtedness for many valuable suggestions obtained from the laboratory notes prepared for the use of medical students by Professor William J. Gies, of Columbia University, and from many standard texts on organic chemistry.

MATTHEW STEEL.

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PRACTICAL COURSE
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CHAPTER

QUALITATIVE ANALYSIS OF ORGANIC COMPOUNDS

1. The Detection of Carbon and Hydrogen. Dry a clean test-tube in a gas flame. Fit it with a cork through which passes a glass tube, bent at a right angle. Mix in a mortar a little dry cane sugar and ten times as much dry CuO; pour this mixture into the test-tube; cork; dip the outside end of the glass tube into baryta solution contained in another test-tube. Heat the sugar mixture over a flame.

Write answers to the following questions in your notebook:

a. What causes the drops of water which are condensed on the cool part of the glass test-tube and tube?

b. To what is the cloudiness which develops in the baryta solution due?

c. Write all equations involved.

2. Detection of Nitrogen in an Organic Substance. *a. As ammonia.* Grind a small portion of the substance under examination (e.g., about ½ gram of casein) with about 2 grams of soda-lime. Place the mixture in a dry test-tube and heat. Note the odor of the fumes that are given off. If the odor of ammonia is not distinct, moisten a piece of red litmus paper in distilled water and hold it in the fumes. It should turn blue.

b. As sodium cyanide. (*Lassaigne's method.*) By means of a clamp fix a small, dry, clean test-tube in a vertical position and introduce into it a piece of freshly cut metallic sodium about the size of a pea. Gently heat the tube until the sodium has melted and begun to vaporize. Then add in minute portions the substance to be tested (e.g., dried egg-albumin). Continue the heating for about half a minute; cool the tube; break the lower end of it in a mortar containing 2 c.c. of alcohol; and, finally, add about 10 c.c. of water as soon as effervescence has ceased. (Care must be taken at this point, as small pieces of sodium are sometimes left unattacked by the alcohol and are liable to be thrown into one's face.)

Add 5 drops of dilute sodium hydroxide solution to the liquid in the mortar and filter. Place a portion of the filtrate, which should be clear and colorless, in a test-tube and add 2 drops of a freshly prepared solution of ferrous sulphate. Wave the tube gently back and forth in the flame until it comes to a boil, or until the brown films of ferric hydroxide appear on the side of the tube.

Cool by holding the tube under the tap water; then add dilute sulphuric acid, drop by drop, until the ferric hydroxide dissolves. If the acid solution is distinctly blue, nitrogen is present.

Write answers to the following questions in your notebook:

a. What is accomplished by heating the nitrogenous compound with sodium?

b. Why is sodium hydroxide solution added to the solution of the melt?

c. What is accomplished by the digestion with ferrous sulphate?

d. Why is oxidation of the iron salt permitted?

e. Write all equations involved.

3. Tests for Halogens. *First method. Beilstein's test.* Loop a piece of copper wire and heat the looped end in a Bunsen flame until the flame is no longer colored green.

A little of the substance to be tested, e.g., chloroform, is placed on it and is heated again. Explain the phenomena observed.

If this test yields a positive or doubtful result, try the following method:

Second method. Decompose some of the substance to be tested for halogens by means of sodium in the same manner as described in the test for nitrogen. In this case make the solution just acid with nitric acid, bring to a boil and allow to cool. Divide the solution into three parts. To one add a few drops of silver nitrate solution. To the second add ½ c.c. of carbon bisulphide and shake; then add 2 drops of chlorine water and shake again. To the third portion add a little carbon bisulphide and potassium nitrite solution and shake.

Write answers to the following questions:

a. What products are formed by the action of sodium upon the substance?

b. Why is the solution acidified with nitric acid and boiled?

c. How much (and how little) does the formation of a precipitate with silver nitrate indicate?

d. What is the action of chlorine water upon a solution of the sodium halides. Of potassium nitrite?

e. Of what use is the carbon bisulphide?

4. Test for Sulphur. *As sodium sulphide.* Decompose a little of the substance with sodium in the same manner as in the test for nitrogen. To a little of the filtered solution add 3 drops of a 1 per cent solution of sodium nitroprusside. To another portion add 1 c.c. of a solution prepared by adding sodium hydroxide to a solution of lead acetate until the precipitate first formed just redissolves. To a third portion add ½ c.c. of silver nitrate solution. Write all reactions involved.

As sulphate. Fuse a small portion of the substance provided (dried egg albumin) in a crucible with three times its quantity of fusion mixture ($2KNO_3, Na_2CO_3$). Heat cau-

tiously at first round the edge and continue heating until all charred particles have vanished. Cool; extract with hot water, and test the filtered solution for sulphates with barium chloride in the presence of a mineral acid (HCl). Write reactions involved.

5. Test for Phosphorus. Fuse, as described for sulphur, some casein with fusion mixture. Extract the fused mass with hot water and divide the solution into two parts. To the one part add excess of nitric acid and ammonium molybdate and warm; a yellow precipitate indicates phosphoric acid; to the other part add excess of ammonia and test for phosphates with magnesia mixture. Write all equations.

CHAPTER II

PURIFICATION OF ORGANIC SUBSTANCES

6. Separation of a Compound by Precipitation and its Purification by Washing and Recrystallization. To a given solution of sodium benzoate add a slight excess of dilute hydrochloric acid. Benzoic acid is precipitated. Filter off the precipitate and wash it free from hydrochloric acid with water. If possible the filtration should be done on a Buchner funnel. Then place the substance in a small beaker and dissolve in boiling water. Use just sufficient water to dissolve it. Filter the hot solution through a folded filter paper into a clean beaker. Cover the beaker with a watch-glass and allow to cool. Pure benzoic acid will crystallize out. Filter off the crystals, wash them with a little cold water, and then dry the crystals thoroughly between sheets of filter paper. Test the purity of your product by estimating its melting-point. (See Exp. 7.)

7. Determination of the Melting-point of Solids. Pulverize your own product of benzoic acid and introduce a small quantity, sufficient to occupy a length of 1 cm., into a melting-point tube. This consists of a thin-walled glass tube about 1 mm. in diameter and 5–6 cm. long and closed at one end. It is made by heating a dry piece of glass tubing and when soft drawing it out; the long capillary tube thus made is cut into the required lengths and one end is sealed by holding it in the flame. Attach the melting-point tube, when filled, by a narrow rubber band to a thermometer. Suspend the thermometer by means of a clamp in a beaker of concentrated sulphuric acid or cottonseed oil which is placed upon a wire gauze or sand-bath on a tripod. Gradually heat the beaker and at the same time stir the liquid with a glass stirrer. Note the temperature at which the substance melts.

NOTE.—A pure solid organic compound melts sharply at a definite temperature. An impure organic compound does not melt sharply and it melts at a lower temperature than a pure compound. The melting-point of a compound helps in its identification.

8. Separation of Two or More Solids by Means of Selective Non-miscible Liquids. If two solids, for example, are mixed together, and we desire to separate them, the task is easy, provided one is soluble in a liquid which is non-miscible with another liquid in which the other solid is soluble.

To separate succinic acid and urea: Place an aqueous solution of succinic acid and urea in a separatory funnel and add about one-quarter its volume of ether. Shake up the two solvents. (An increase in pressure usually occurs when ether and water and air are first shaken together in a separatory funnel. While shaking, therefore, the top of the funnel must be occasionally opened after allowing the liquids to settle at the other end.) After thoroughly shaking, allow the liquids to separate from one another, remove the stopper from the funnel and run the heavier liquid (water) into a clean beaker. Then pour the ether layer into a clean basin and allow it to evaporate.

Extract the aqueous layer two or three times with fresh quantities of ether. Combine the ethereal extracts and allow them to evaporate. Succinic acid will be obtained. Recrystallize it from hot water and determine its melting-point.

Evaporate the aqueous solution on the water-bath; urea remains. Recrystallize it from hot water and determine its melting-point.

9. Purification of Liquids by Distillation. A pure liquid has a constant boiling-point; hence, by distilling a mixture and carefully watching the boiling-point, we can separate one liquid from a mixture of two or more liquids, and by redistilling the distillate that comes over at approximately the right boiling-point we can obtain a comparatively pure product.

Make a mixture of 200 c.c. each of common commercial alcohol and water. Determine the specific gravity of the mixture, and by means of hydrometers or alcoholometers determine the percentage content of alcohol. Transfer the liquid to a distilling flask (a round-bottom flask with a side-tube in its neck), filling it half or at most two-thirds. Close the neck with a cork which carries a thermometer and adjust it so that the bulb of the thermometer is just below the opening of the side tube and not touching the walls. Connect the side tube by means of a cork to a clean, dry condenser, and place a receiver (flask) at the other end of the condenser. Place the distilling flask on a water-bath, gently heat the liquid and observe the thermometer. The mercury rises rapidly at first and then becomes stationary at a definite temperature. This is the boiling-point of the liquid. Pure alcohol boils at 78.5° C. In a mixture of alcohol and water the first fixed boiling-point will be from about 78 to 80° C. Collect the distillate as long as the thermometer remains between these two points. As soon as the thermometer rises above 80° C., remove the receiving flask; we shall call this distillate *A*. If it is desired to get all the alcohol, adjust another receiving flask and continue the distilling of the mother liquor. To obtain pure alcohol from distillate *A*, add several lumps of fused calcium chloride and allow it to stand for 12 to 24 hours. The calcium chloride has a great affinity for water and will remove it from the alcohol. Filter this liquid into a small dry, clean distilling flask and distil again. This time most of the liquid should distil over at 78.5° C. To ascertain whether any water still remains in the alcohol, transfer a small amount to a test-tube and add about $\frac{1}{2}$ gram of fused copper sulphate. Fused copper sulphate is a dirty white in color, but if it comes in contact with water it will become blue. Prove this latter by mixing $\frac{1}{2}$ c.c. of water with 4 c.c. of alcohol and then adding $\frac{1}{2}$ gram of fused copper sulphate.

CHAPTER III

THE ALIPHATIC HYDROCARBONS

A. The Paraffins

10. Preparation and Properties of Methane. Mix together 2 grams of fused sodium acetate and 6 grams of soda lime. Place the mixture in a test-tube and heat. Marsh gas is evolved and may be ignited at the mouth of the test-tube (dry distillation).

11. Fractional Distillation of Kerosene. Place about 50 c.c. of refined petroleum in a small, dry, distilling flask; connect it with a condenser and distil. Notice that the temperature indicated by the thermometer never remains constant for any length of time. This shows that the liquid is a complex mixture. Collect several fractions boiling within 10° ranges of temperature in separate receivers. By redistilling these fractions in the same way, pure products may eventually be obtained.

12. Inflammability of Kerosene. To about 3 c.c. of refined petroleum in a porcelain dish place a lighted match. What happens? Now warm the liquid on a water-bath to 40° C. and again apply a lighted match. Now what happens?

13. Inertness of the Saturated Hydrocarbons. Shake up some petroleum ether with:

A. Some concentrated sulphuric acid.

B. Some concentrated nitric acid.

C. Some sodium hydroxide solution.

Repeat the above experiment using paraffin wax instead of petroleum ether.

What happens in each case?

What do you conclude in regard to the paraffins from these experiments?

B. The Unsaturated Hydrocarbons

14. Preparation of Ethylene. C_2H_4. Set up an apparatus consisting of a 1-liter flask, a condenser, two gas wash bottles, and a pneumatic trough, making the connections as follows: Select a snugly fitting cork for the flask, and bore two holes in it. Into one hole insert a thermometer so that it will come within an inch of the bottom of the flask. Into the other hole insert a bent glass tube. Connect this tube with the condenser, which in turn is attached to a tube which passes just through the stopper of the first gas wash bottle. Connect the latter with the second wash bottle by means of a bent glass tube which reaches nearly to the bottom of each.

Pass another tube from just below the stopper of the second wash bottle to the pneumatic trough. The first wash bottle is to be left empty, and the second one is to be half filled with a concentrated solution of sodium hydroxide. This arrangement is necessary to prevent back-suction of the alkali into the hot acid solution.

When all the apparatus has been carefully adjusted, pour 30 c.c. of absolute alcohol into the flask, add a spoonful of sand, and then add, in small quantities at a time and with constant shaking, 100 c.c. of concentrated sulphuric acid. If the mixture should become very warm during this process, cool the flask under running water before adding more acid. Finally, connect the flask with the condenser and heat cautiously over a wire gauze until the temperature reaches 180°. Adjust the flame so that this temperature may be kept fairly constant. When a sample of gas collected in a test-tube over water burns readily, fill several 250 c.c. wide-mouthed glass-stoppered bottles with the gas.

Of what use was the sodium hydroxide solution in the wash bottle?

15. Properties of Unsaturated Hydrocarbons. *a. Inflammability of ethylene.* Turn one of the bottles mouth upwards, and stopper it with a cork containing two holes.

In one hole insert a short glass tube, nearly closed at the outward end. In the other hole insert a tube long enough to extend to the bottom of the bottle. Connect this tube, by means of a piece of rubber tubing, to a water bottle, and allow a stream of water to flow in slowly. At the same time ignite the gas which escapes from the other tube. Note the luminosity of the flame.

b. Action of bromine on ethylene. Pour 1 c.c. of bromine water into one of the bottles. Close the bottle with a glass stopper, and agitate the contents. Note the disappearance of the red color, and the appearance of oily drops on the sides of the bottle. On opening the bottle, notice the characteristic odor of the new compound. What is this substance? Write the equation of the reaction.

c. Von Baeyer's test for the double bond. Into another of the bottles of ethylene pour 2 c.c. of very dilute potassium permanganate solution and 1 c.c. of a 5 per cent sodium carbonate solution. Stopper the bottle with a glass stopper and shake it. Note the changes in color. What new compound is produced from the ethylene? Write the equations of the reaction.

d. Unsaturated compounds in illuminating gas. By means of a rubber tube attached to a gas jet, fill two bottles with gas by displacement of water. Repeat experiments *b* and *c*. Are unsaturated substances present?

e. Action of bromine on amylene. Dissolve 1 c.c. of amylene in 5 c.c. of carbon tetrachloride in a test-tube, and add slowly and with constant shaking, a 1 per cent solution of bromine in carbon tetrachloride. Does the red color disappear?

f. Action of oxidizing agents on amylene. Mix 10 drops of amylene with 5 c.c. of a 5 per cent solution of sodium carbonate. Then add, drop by drop, with constant shaking, a 1 per cent solution of potassium permanganate. What changes occur?

16. Preparation and Properties of Acetylene. C_2H_2. Support a dry 300-c.c. distilling flask by means of a tripod

and a clamp. Connect the side-arm of the flask with a bent glass tube leading just through the stopper of an empty bottle. Insert another bent glass tube through the stopper, to the bottom of this bottle, and connect it with a bent delivery tube which dips under water. Adjust a dropping funnel in the neck of the flask by means of a hole in the stopper. Place about 10 grams of calcium carbide in the flask, and allow water to enter from the dropping funnel, *drop by drop*. Collect four or five 250-c.c., glass-stoppered, wide-mouthed bottles of gas. Discard the first one, as it contains impurities.

a. With the acetylene gas collected in the other bottles, perform the tests described in experiment 15, *a*, *b*, and *c*.

Record your results. Write all equations.

b. Test for the triple bond. Prepare an ammoniacal solution of cuprous chloride as follows: Dissolve 3 grams of copper oxide in 10 c.c. of concentrated hydrochloric acid and 10 c.c. of water. As soon as the oxide has dissolved add 3 grams of copper filings and boil the mixture until the solution becomes colorless.

Cool, and then decant off about one-fourth of the liquid and render it alkaline with ammonium hydroxide. Pour 5 c.c. of this liquid into one of the bottles containing acetylene gas, and shake. A red precipitate of copper acetylene, C_2Cu_2, is formed. To a small portion of this precipitate add a drop or two of HCl and notice the odor of acetylene.

CHAPTER IV

HALOGEN DERIVATIVES OF THE HYDROCARBONS

17. Preparation of Chloroform. Into a 300-c.c. flask pour 150 c.c. of a saturated solution of fresh bleaching powder (prepared in advance for the class). Connect the flask, by means of a bent tube, to a condenser and receiver. Also suspend a dropping funnel through the cork. Into the funnel pour 10 c.c. of acetone and 20 c.c. of water, run this into the flask very slowly, shaking frequently. Heat the flask as long as droplets of chloroform appear with the water at the condenser. Transfer the distillate to a separating funnel, shake with several portions of water. Run the chloroform into a flask, add fused calcium chloride, cork, and let it stand until the next period. As the yield is small, it need not be redistilled.

Write all reactions involved.

18. Properties of Chloroform. Shake a small quantity of chloroform with water in a test-tube and add a few drops of silver nitrate solution. Does any precipitate occur?

Repeat with old chloroform. Now what do you observe?

What changes take place in moist chloroform when it is exposed to air and light?

19. Preparation of Iodoform. Dissolve 10 grams of potassium carbonate in 75 c.c. of water, add 15 c.c. of alcohol. Warm on a water-bath to about 70° C. Add, in small quantities at a time, 5 grams of iodine. Stir the mixture after each addition. Finally add more potassium carbonate, if any color of iodine remains. After all the iodine has dissolved, cool, filter, and wash with water. Dissolve the iodoform in a few c.c. of warm alcohol, and purify by recrystallization. Examine the crystals under the micro-

scope, filter and dry between filter-papers. Make sketch of crystals in your note-book. Test the solubility of your iodoform crystals in ether, alcohol and water.

What is produced on boiling iodoform with an aqueous solution of sodium hydroxide?

CHAPTER V

ALCOHOLS

20. Preparation of Ethyl Alcohol by Fermentation. Dissolve 20 grams of commercial glucose in 100 c.c. of water and add 10 c.c. of a nutrient solution. (The yeast requires the presence of certain inorganic salts for its nutriment. A suitable solution may be prepared by dissolving 10 grams each of potassium nitrate, magnesium chloride, calcium nitrate, and potassium phosphate in water and diluting to 1 liter.) Place the solution in a flask which it will fill almost to the top, and which is provided with a Bunsen valve. (A Bunsen valve consists of a short piece of glass tubing passing through the cork and connected at its outer end with a piece of small rubber tubing about 2 inches long, whose other end is closed by means of a piece of glass rod. In the rubber tubing is a longitudinal slip about half an inch long which permits the egress but not the entrance of gases.) Finally macerate half a yeast cake with water, and add the resulting paste to the solution. Stopper the flask tightly and allow the mixture to stand until the next exercise. Finally, distil off 5 c.c. of the fermented sugar solution, and make the iodoform test for alcohol in the distillate.

21. Preparation of Absolute Alcohol. Put 100 c.c. of 95 per cent alcohol in a perfectly dry flask. Add 40 grams of fused calcium oxide in small lumps and tightly stopper the flask. Allow the mixture to stand until the next exercise. Decant off and distil the alcohol which has been dried over calcium oxide, using a perfectly dry condenser and receiver. Test the distillate for water with anhydrous copper sulphate, noting the time necessary for the appearance of a blue coloration. Prepare the anhydrous copper sulphate by heating two or three grams of pulverized crys-

talline copper sulphate carefully in an evaporating dish until it turns white. Avoid overheating, since this causes the formation of black copper oxide.

22. Properties of Ethyl Alcohol. *a. Action of metallic sodium.* To 10 c.c. of absolute alcohol in a small flask add about 0.5 gram of metallic sodium. Observe that effervescence occurs, just as it does when metallic sodium is added to water but that the reaction is not nearly so violent. Collect the gas evolved in an inverted tube and show that it is hydrogen by burning it. When the sodium has dissolved, evaporate the solution to dryness on a water bath. A white solid—sodium ethylate or sodium alcoholate—remains, which is very hydroscopic, and is decomposed by water, yielding alcohol and sodium hydroxide. Dissolve a little of your sodium alcoholate in a few c.c. of water. Neutralize carefully with hydrochloric acid; then evaporate to dryness. Ignite, cool and test the residue.

What is this residue?

Write all equations involved.

b. Oxidation. To a little dilute alcohol in a test-tube add a few drops of potassium bichromate solution and a little dilute sulphuric acid; warm. Note the characteristic odor of aldehyde.

Observe that the solution turns green. Why?

Write reactions involved in the above oxidation.

c. Iodoform test for ethyl alcohol. To 1 c.c. of alcohol add 5 c.c. of water, then add 2 c.c. of a 3 per cent solution of iodine in potassium iodide and 10 drops of a 5 per cent sodium hydroxide solution. After shaking, warm the mixture for a few seconds in a beaker of water heated to about 70° C.

Why is it not advisable to heat the solution above 70° or to add an excess of sodium hydroxide? Compare with Exp. 19.

d. Action of phosphorus pentachloride. To a small quantity of alcohol add a few drops of phosphorus pentachloride. A vigorous action occurs and hydrochloric

fumes are evolved. This experiment must be performed under the hood.

What are the other products produced?

What *radical* is indicated by the phosphorus pentachloride reaction?

Write reactions.

23. Determination of the Quantity of Alcohol in an Aqueous Solution. A dilute alcoholic solution containing certain non-volatile impurities will be supplied for this test.

Place 200 c.c. of the alcoholic solution in a distilling flask and distil until all the alcohol has come over. This will necessitate collecting at least 100 c.c. of distillate, and the residue should be boiling steadily at about 100°. Make the distillate up to the original volume with distilled water. Determine the specific gravity with a hydrometer, and from these data calculate the percentage by volume of alcohol in the original mixture.

Define percentage by volume and percentage by weight.

CHAPTER VI

THE ETHERS

24. Preparation of Ethyl Ether by the Continuous Process. A distilling flask of about 300 c.c. capacity is fitted with a cork carrying a tap funnel and a thermometer, both arranged so as nearly to touch the bottom. After 50 c.c. of 95 per cent ethyl alcohol and 50 c.c. of concentrated sulphuric acid have been cautiously mixed in the flask, 5 grams of anhydrous aluminium sulphate are added, and the flask is connected to a long condenser. An adapter is fitted to the lower end of the condenser and passes through the neck of a suction flask, which is surrounded by ice and has a piece of rubber tubing attached to its side arm. The rubber tubing is arranged so as to carry any volatilized ether into the waste pipe of the sink. Heat is then applied until a temperature of 135 to 140° is attained. As soon as the distillation of ether begins, alcohol is introduced from the tap funnel at the same speed as the liquid distils (about 3 drops a second), and the distillation continued with a small flame so as to maintain a constant temperature. After about 100 c.c. of distillate has been obtained it is agitated in a separatory funnel with an equal volume of water, which dissolves most of the admixed alcohol and sulphurous acid, leaving a layer of ether floating on the surface of the solution. After the ether is separated from the aqueous liquid, it is dried by agitation for about ten minutes with fused, powdered calcium chloride.

How would you test for the presence of water in a given sample of ether?

Write reactions involved in the preparation of ether by this process.

How else could ether be made?

How would you make a mixed ether?

25. (Demonstration.) Preparation of Anhydrous Ether. Ether obtained by a method similar to that indicated in Exp. 24 contains traces of alcohol and water. These traces can be removed only by treatment with metallic sodium.

The ether is placed in a flask provided with a calcium chloride tube to prevent the access of moisture, and to allow the escape of hydrogen; several pieces of freshly cut metallic sodium are added and the mixture is repeatedly agitated until no further effervescence is observed. The ether is decanted into a perfectly dry distilling flask and distilled from a water-bath. Pure ether of constant boiling-point (35°) is collected.

Properties of Ether

26. Miscibility of Ether with Other Liquids. Into each of six perfectly clean, dry test-tubes place 5 c.c. of ethyl ether, then mix with an equal volume of the following: Alcohol; glycerol, benzene, chloroform, petroleum ether, and water.

Record your results.

27. Solvent Power of Ether. Add about 5 c.c. of ethyl ether to each of five test-tubes containing a lump about the size of a pea of (1) lard, (2) paraffin, (3) hard-boiled egg-white, (4) hard-boiled egg-yolk, (5) bread crumbs. Warm each test-tube by holding it in your hand for a couple of minutes; then if you have any doubt in regard to the solubility of the substance, filter the supernatant ether through a dry filter paper onto a clean dry watch-glass and ascertain if any residue remains after the ether has evaporated.

Record your results.

28. Absorption of Heat Caused by Evaporation of Ether. Demonstrate that the evaporation of ether causes absorption of heat by placing a small quantity in the palm of the hand, and blowing on it. A sensation of cold is felt immediately.

29. Inertness of Ether Towards Chemical Reagents. Into each of 5 test-tubes place 5 c.c. of *anhydrous* ether and shake up with the following:

a. 2 c.c. of phosphorus pentachloride.
b. 2 c.c. of 10 per cent sodium hydroxide solution.
c. 2 c.c. of 10 per cent alcoholic soda.
d. 2 c.c. of concentrated sulphuric acid.
e. A lump the size of a pea of metallic sodium.

What happens in each case?

What do you conclude in regard to the chemical activity of ethers from these experiments?

CHAPTER VII

THE ALDEHYDES AND KETONES

30. Preparation of Formaldehyde. *a.* Heat a few drops of methyl alcohol with potassium bichromate and sulphuric acid. Note the pungent, suffocating odor of formaldehyde. Hold a piece of blue litmus paper in the fumes.

What do you observe? Why?

b. Take a piece of fine copper wire about a foot long and twist one end of it into a spiral by winding about a glass stirring rod. Heat the spiral to redness for a couple of minutes. Cool. Note that it is nearly black due to the oxidizing of the copper. Heat the spiral again to redness and at once plunge it into a test-tube containing about 2 c.c. of methyl alcohol. The copper oxide is reduced to metallic copper (note change of color). The vapors of formaldehyde are especially noticeable.

Ascertain if the fumes are acid.

Write the equations for the reactions.

31. Detection of Formaldehyde in Milk. (Leach's method.) Put 10 c.c. of the milk to be tested into a casserole or small porcelain dish and add 10 c.c. of concentrated hydrochloric acid which contains one drop of a 5 per cent solution of ferric chloride. Hold the casserole just above a small Bunsen flame, and keep it moving in a rotary fashion until the liquid just reaches the boiling-point. Should formaldehyde be present, a violet color, more or less pronounced, will appear when the temperature reaches 80° or 90°. In the absence of formaldehyde, the liquid will assume a turbid yellowish-brown hue.

32. The Resorcinol Test for Formaldehyde. To about 2 c.c. of *very dilute* formaldehyde solution (or solution suspected of containing a small amount of formaldehyde), add

one drop of a 1 per cent aqueous solution of resorcinol and shake thoroughly. Pour this liquid cautiously into a second test-tube containing about 3 c.c. of concentrated sulphuric acid. If the second test-tube is properly inclined the mixture will form a distinct layer upon the surface of the acid.

A red zone slightly violet in color will appear, and above the zone there will be a light flocculent precipitate. This reaction is characteristic of formaldehyde; other aldehydes do not show this behavior.

33. (Demonstration.) **Action of Formalin on Proteins.** The yolk of an egg is carefully placed in formalin (a 40 per cent solution of formaldehyde in water). On allowing to stand for some time, it will be found to have acquired the consistency of a rubber ball.

34. Preparation of Acetaldehyde. In a 300-c.c. distilling flask place 20 c.c. of 95 per cent ethyl alcohol, 100 c.c. of dilute sulphuric acid (1 : 10), and 40 c.c. of a 10 per cent solution of potassium bichromate, attach to a condenser so that the receiving end dips just under the surface of about 10 c.c. of cold water. Distil until 15 or 20 c.c. have been collected. This distillate may be used to perform the aldehyde reactions described in Exps. 35, 36, 37, 39, 40, 41 and 42.

Properties of Aldehydes

35. Silver Mirror Test. To a little silver nitrate solution add dilute ammonium hydroxide solution until the precipitate which first formed just redissolves. Then add a few drops of dilute aldehyde solution (if the distillate obtained in Exp. 34 is used, first dilute a small portion of it with water), place in a water-bath containing cold water and heat to boiling. A mirror of metallic silver forms on the glass.

NOTE.—To obtain good results the test-tube used in this experiment must be *perfectly* clean. The best way to clean the test-tube is to boil a little sodium hydroxide in it, and then to wash several times with water.

36. Reduction of Fehling's Solution. To a little mixed Fehling's solution add a few drops of dilute aldehyde solution and warm. A red or yellow precipitate of cuprous oxide is formed. Explain these changes.

NOTE.—Fehling's solution consists of copper sulphate, caustic soda, and Rochelle salt (sodium potassium tartrate). When caustic soda is added to copper sulphate a blue precipitate of cupric hydrate, $Cu(OH)_2$, is formed, which turns black on boiling. The presence of the Rochelle salt keeps the $Cu(OH)_2$ in solution, and forms a deep blue solution. This solution does not keep, so that it must be freshly made for each experiment. For this purpose the two solutions must be kept separate. One contains the copper sulphate, the other the Rochelle salt and caustic soda. When required for use, equal parts of each are mixed together.

37. Formation of Aldehyde Resin. To a little acetaldehyde solution add sodium hydroxide and warm. The liquid becomes darker and a brown resinous substance, aldehyde resin, separates out, with the evolution of a peculiar smell. Aldehyde resin is insoluble in water, but soluble in alcohol and ether.

38. Repetition of Aldehyde Resin Test with Formaldehyde. Repeat Exp. 37, using formaldehyde instead of acetaldehyde. Do you get the same result?

39. Schiff's Aldehyde Reaction. To a little dilute acetaldehyde solution add a solution of magenta decolorized with sulphurous acid (Schiff's aldehyde reagent). The red color of the dye is restored.

40. Oxidation of an Aldehyde to an Acid. Mix 5 c.c. of 10 per cent potassium bichromate, 5 c.c. of dilute sulphuric acid, and a little of the aldehyde solution and warm. The solution becomes green. Why? Hold a piece of blue litmus in the steam. Does it turn red? Write reaction. If acetaldehyde is used, what acid is obtained?

41. Polymerization of Aldehydes. Place a clean test-tube, containing about 2 c.c. of acetaldehyde, in a freezing-mixture (2 parts cracked ice and 1 part sodium chloride). Then add a small drop of concentrated sulphuric acid.

This can best be done by dipping a clean stirring-rod into the acid. On stirring the thoroughly cooled aldehyde, it should finally solidify. Is the odor still like that of aldehyde? Explain this change.

42. Formation of Hydrazone. Dissolve about a gram of a mixture of phenylhydrazine hydrochloride and sodium acetate (these are mixed on a basis of 1 part of the phenylhydrazine hydrochloride to 2½ parts of sodium acetate) in 10 c.c. of water, add 10 drops of acetaldehyde and warm in a water-bath; an oil (acetaldehyde phenylhydrazone) is formed. Write equation of the reaction.

43. Reducing Action of Chloral and Chloral Hydrate. Repeat Exps. 35 and 36, using a solution of chloral hydrate instead of the aldehyde.

What changes occur? Explain them.

Write equations showing how chloral could be prepared from acetaldehyde; from alcohol.

44. Formation of Chloroform from Chloral Hydrate. To 5 c.c. of a 50 per cent aqueous solution of chloral hydrate add about 2 grams of solid sodium hydroxide and warm gently (with hand). Identify the liquid which separates. Write equation of the reaction.

THE KETONES

45. Preparation of Acetone. Place in a round-bottom flask 50 grams of dry calcium acetate. Connect the flask with a condenser and heat on a sand-bath to a high temperature. Acetone is the principal ingredient of the distillate.

Write equation. Use this distillate in Exps. 46 to 52.

Properties of Ketones

46. Oxidation of Acetone. Mix 2 c.c. of the acetone distillate with a small quantity of very dilute sulphuric acid. Heat gently and add potassium permanganate solution, little by little, until the warm solution retains a pink

color. Filter, acidify strongly with sulphuric acid and distil. Since the quantity is small, a test-tube fitted with cork and L-shaped glass tube may be used instead of a distilling flask. Retain the distillate until after you perform the experiments on acetic acid (Exp. 56), then ascertain whether the distillate contains acetic acid.

47. Reduction of Acetone. Repeat Exps. 35, 36 and 39, using acetone, then answer the following questions: *a.* Does acetone give a positive silver mirror test? *b.* Does it reduce Fehling's solution? Does it color Schiff's reagent? What would be formed by the reduction of acetone?

48. Formation of Addition Products. Shake thoroughly a mixture consisting of 5 c.c. of a saturated solution of sodium hydrogen sulphite and 5 c.c. of the acetone distillate. Cool. Examine the crystalline deposit with a microscope.

Do all ketones and aldehydes form similar addition products with sodium hydrogen sulphate?

49. Formation of Hydrazones by Ketones. Repeat Exp. 42, using acetone instead of acetaldehyde. Does acetone form a hydrazone?

50. Sodium Nitroprusside Test. To about 5 c.c. dilute acetone solution (or solution suspected of containing acetone), add a few drops of a freshly prepared aqueous solution of sodium nitroprusside and about 2 c.c. of 8 per cent sodium hydroxide solution. A ruby red color is produced if the acetone is present. Acidify with acetic acid. Is there any change of color?

51. Salicylic Aldehyde Test. To 10 c.c. of dilute acetone solution (or solution suspected of containing acetone), add about a gram of solid sodium hydroxide, and before it dissolves introduce 10 drops of salicylic aldehyde. Warm to 70° in a water-bath. A purple-red ring appears if acetone is present.

NOTE.—If the alkali has dissolved before adding the salicylic aldehyde the liquid becomes yellow, red, and finally purplish red.

52. Iodoform Test for Acetone. To about 5 c.c. of dilute acetone solution (or solution suspected of containing acetone), add about 10 drops of sodium hydroxide and then, drop by drop, iodine solution, iodine in potassium iodide, until the liquid is faintly yellow. If acetone is present iodoform will separate at once.

Note.—Acetone yields iodoform at room temperature, whereas alcohol requires heating.

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CHAPTER VIII

FATTY ACIDS

Formic Acid. Exactly neutralize about 10 c.c. of formic acid with sodium hydroxide and use the solution of sodium formate in Exp. 53 and 54.

53. Reducing Action of Formic Acid. *a.* Mix about 3 c.c. of neutral sodium formate solution with an equal volume of silver nitrate solution and warm. A black deposit of metallic silver is formed. Test the reaction of the filtrate.

Write the equations.

Explain the reducing action of formic acid.

b. To another portion of the sodium formate solution add a few drops of mercuric chloride solution, and warm gently. The white precipitate which separates is mercurous chloride ($HgCl$). Filter the solution; then moisten the precipitate on the filter paper with ammonium hydroxide. Note the change of color. Explain it.

54. Action of Concentrated Sulphuric Acid on Formic Acid. Evaporate about 5 c.c. of the sodium formate solution to dryness. Transfer the solid residue to a dry test-tube; add a little concentrated sulphuric acid and heat. What is the gas evolved? Does it burn?

Write the equation.

55. Preparation of Acetic Acid by Oxidation of Ethyl Alcohol. Ten grams of potassium dichromate are mixed with 10 c.c. of concentrated sulphuric acid in a 300-c.c. distilling flask. The flask is then connected to a condenser by its side arm and 25 c.c. of a 5 per cent aqueous solution of alcohol is added, drop by drop, from a tap-funnel. After all the alcohol has been added, the tap-funnel is closed, the flask is heated, and the distillate collected.

The presence of acetic acid in the distillate may be as-

certained by its odor, and after careful neutralization with sodium carbonate, by the ferric chloride test (Exp. 56).

56. The Basic Acetate Test for Acetic Acid. Neutralize a little dilute acetic acid with sodium hydroxide solution and add a few drops of ferric chloride. The solution turns blood-red, due to the formation of a complex salt known as aceto-ferric acetate. Divide this solution into two parts. Boil part one, and add hydrochloric acid to part two.

Explain the changes observed.

57. Action of Sodium Acetate on Reducing Agents. Repeat Exp. 53, using sodium acetate instead of sodium formate. Does acetic acid have any reducing action?

58. Freezing-point of Glacial Acetic Acid. Immerse a small test-tube containing about 5 c.c. of glacial acetic acid in some freezing-mixture (2 parts ice, 1 part salt). Stir with a glass rod and when the mass is about half frozen insert a thermometer and note the solidifying temperature of the acid. Pure glacial acetic acid melts at 16.5°. How would the presence of water affect the freezing-point?

59. Inflammability of Acetic Acid Fumes. Apply a flame to some glacial acetic acid. Does it burn? Now heat a few drops in a test-tube and apply a flame to the vapors. Do they burn?

Chemical Properties of the Higher Fatty Acids

60. Solubility of Stearic Acid. Test the solubility of stearic acid in water, cold alcohol, warm alcohol and ether.

Record your results.

61. Formation of Soap. Suspend a small amount of stearic acid in water and add sodium hydroxide drop by drop with constant stirring; it dissolves slowly, forming a clear solution which foams considerably on agitation.

Is a sodium soap a hard or soft soap?

62. The Reaction of Soaps. Make a concentrated solution of a neutral soap. Test its reaction to phenolphthalein. Pour some of this soap into a beaker full of distilled water. Explain the changes observed.

CHAPTER IX

ACID CHLORIDES, ACID ANHYDRIDES, ESTERS AND MERCAPTANS

Properties of the Acid Chlorides (Acyl Haloids)

63. Action of Water on Acetyl Chloride. Place 5 c.c. of water in a test-tube and add about ½ c.c. of acetylchloride, drop by drop. What gas escapes? Test the reaction of the liquid that remains in the test-tube with litmus paper. Write the equation for the reaction.

64. Action of Alcohol on Acetyl Chloride. Place 2 c.c. of absolute alcohol in a small test-tube. Hold the test-tube in a beaker of cold water, then add, drop by drop, about 1 c.c. of acetyl chloride. Make the solution faintly alkaline, then pour it upon a watch-glass and notice the odor. What is formed? Write the equation for the reaction.

For the use of acyl haloids as a means of detecting the number of hydroxyl radicals in a carbohydrate see Chapter XII.

Why do the acyl haloids fume in the air?

Properties of the Acid Anhydrides

65. Action of Water on Acetic Anhydride. Mix a few drops of acetic anhydride with a little water. What is formed? How could you detect it?

66. Action of Alcohol on Acetic Anhydride. To about 2 c.c. of absolute alcohol add an equal volume of acetic anhydride, drop by drop, and warm gently in a water-bath. Add a little water to the product and make it slightly alkaline with sodium hydroxide. Pour it upon a watch-glass and notice the odor. Does it resemble the odor obtained by the action of alcohol on acetyl chloride?

Write the equation of the reaction. Do acid anhydrides fume in the air?

Esters of Inorganic Acids

67. Preparation of Ethyl Nitrite. (Sweet Spirits of Nitre.) Warm a little alcohol with copper filings and a few drops of concentrated nitric acid in a test-tube and note the odor of ethyl nitrite. Write the equation of the reaction.

68. Preparation of Potassium Ethyl Sulphate. Carefully mix 10 c.c. of concentrated sulphuric acid with 25 c.c. of absolute alcohol in an Erlenmeyer flask on a water-bath under a reflux (upright) condenser for ½ hour. Cool and then pour into a casserole containing about 150 c.c. of water. Gradually add calcium or barium carbonate, with constant stirring, until the solution is alkaline to litmus. What salt is formed? Boil the mixture and filter. Place the clear filtrate on the water-bath, heat again, and add a strong solution of potassium carbonate until no further precipitate is formed. Allow the precipitated calcium carbonate to settle and decant the supernatant liquid onto a filter paper. Finally evaporate the filtrate to a very small volume. On cooling, crystals of ethyl potassium sulphate will settle out. Wash the crystals with dilute alcohol, dry between filter papers and keep in a small stoppered vial for future experiments.

Write all equations.

Esters of Organic Acids

69. Preparation of Ethyl Acetate. Mix 1 c.c. of absolute alcohol and 1 c.c. of glacial acetic acid in a test-tube and heat in a boiling water-bath. Note the change of odor. Write the equation of the reaction. Compare Exps. 64 and 66.

70. Hydrolysis (Saponification) of Ethyl Acetate. Place in a distilling flask 20 c.c. of ethyl acetate, 50 c.c. of a 20 per cent solution of sodium hydroxide, and a small piece of pumice stone.

Put the flask on a water bath and attach a condenser in a vertical position so that the condensation products will return to the flask ("reflux condenser"). Shake the mixture frequently to prevent bumping. After the layer of ethyl acetate has disappeared, connect the condenser in the usual manner for distillation, and collect all the liquid that distils under about 90°.

Prove the presence of ethyl alcohol in the distillate by applying the iodoform test (Exp. 22, *c*.)

The presence of acetic acid in the residue in the flask should be proven by acidifying with dilute sulphuric acid and again distilling. This distillate should be tested for acetic acid. Apply the basic acetate test. (Exp. 56.)

Write all equations.

71. Preparation of Ethyl Mercaptan. Prepare 2 to 5 c.c. of a saturated solution of ethyl potassium sulphate from the product prepared in Exp. 68. Add an equal volume of a 33 per cent solution of potassium hydrogen sulphide and warm.

Notice the garlic-like odor of the mercaptan.

What products are formed when the mercaptans are oxidized?

Write equations showing how ethyl mercaptan may be used in the preparation of the following: Sulphonal, trional, and tetronal.

CHAPTER X

AMINES, AMIDES, AND CYANOGEN COMPOUNDS

The Amines

72. Reactions of a Typical Primary Amine. *a. Liberation of the base.* Add a few drops of sodium hydroxide solution to about 5 c.c. of a 5 per cent aqueous solution of methyl amine hydrochloride and warm. Monomethylamine is evolved and may be recognized by its fishy, ammoniacal odor, its reaction to litmus, and its inflammability (unlike ammonia).

b. Action of nitrous acid. Acidify a few c.c. of a solution of monomethylamine hydrochloride with HCl and add some sodium nitrite solution.

What is the gas evolved?

Write the equation of the reaction.

c. Isonitrile reaction. Warm about 2 c.c. of a solution of monomethylamine hydrochloride with a couple of drops of chloroform and about 2 c.c. of alcoholic sodium hydroxide.

Notice the characteristic odor.

Write the equation of the reaction.

d. Reaction with copper sulphate. To about 1 c.c. of a dilute solution of copper sulphate add, drop by drop, some of the monomethylamine hydrochloride solution.

Notice the deep blue color.

73. Reactions of the Secondary Amines. Repeat Exp. 72, using dimethylamine instead of the monomethylamine.

Record your results.

74. Reactions of the Tertiary Amines. Repeat Exp. 72, using trimethylamine instead of the monomethylamine.

Record your results.

The Amides

75. Preparation of Acetamide. Mix about 5 c.c. of ethyl acetate with an equal amount of ammonium hydroxide solution (sp. gr. 0.90), in a small flask. Place this flask in a basin of warm water (about 50° C.) and allow it to stand until the two layers form a homogeneous solution. Finally connect with a long glass tube and distil. At first the ammonia is expelled, also alcohol and water; subsequently the acetamide will distil over, and will solidify.

Write the equation of the reaction.

76. Properties of Acetamide. This experiment may be performed either with the product obtained in Exp. 75 or a purified product supplied by the instructor.

a. Action of nitrous acid. To a little acetamide solution add some sodium nitrite solution and a few drops of dilute hydrochloric acid. Note the effervescence of nitrogen gas.

Write the equation of the reaction.

Compare with Exp. 72 *b*.

b. Hydrolysis by means of an alkali. Boil a little acetamide solution in a beaker with some sodium hydroxide solution. Note the odor of the fumes. Ascertain if ammonia is given off by holding a piece of moist red litmus paper in the fumes.

c. Hydrolysis by means of heating with an acid. Boil a little acetamide solution in a beaker with a few c.c. of dilute hydrochloric acid. Neutralize with sodium hydroxide solution and test for acetic acid with ferric chloride (Exp. 56).

Write all equations of the reactions.

77. Preparation of Carbamide (Urea). Dissolve 20 grams of potassium cyanate in a small volume of water in a casserole. Add to it 20 c.c. of a saturated solution of ammonium sulphate. Evaporate to dryness on a water-bath. (The potassium cyanate first reacts with the ammonium sulphate and forms ammonium isocyanate, and then urea.) Add 25 c.c. of 95 per cent alcohol to the dry residue; stir; place on a water-bath and heat just to the boil-

ing-point; then pour the hot solution on a small dry filter. Evaporate the filtrate to dryness on a water-bath; redissolve the residue in 15 c.c. of absolute alcohol; again filter on a small dry filter; and evaporate this filtrate *nearly* to dryness on a water-bath. On allowing the alcoholic residue to cool, crystals of urea will form.

Write all equations.

78. Reactions of Urea. *a. Solubility.* Test the solubility in water, cold alcohol, and ether. Use small quantities of solute and solvent.

b. Formation of urea nitrate. To a drop of a concentrated aqueous solution of urea add a drop of strong nitric acid. Crystals of urea nitrate are formed. Examine them under a microscope. What shape are they?

c. Formation of urea oxalate. To a drop of a concentrated aqueous solution of urea add a drop of a saturated solution of oxalic acid. Crystals of urea oxalate are formed. Examine these under the microscope. What shape are they?

d. Formation of biuret. Heat some dry crystals of urea to a high temperature in a test-tube. Note carefully what takes place as the heating is continued. Determine the reaction of the vapor produced.

Let the test-tube cool. Examine the residue. Does it resemble urea in appearance?

The white residue consists principally of biuret, but contains also cyanuric acid. Dissolve some of the solid mass in a few c.c. of water, add an equal volume of sodium hydroxide solution, and one drop of a 1 per cent copper sulphate solution. Note the pinkish color that forms (biuret reaction).

e. Repeat the biuret reaction with an aqueous solution of unheated urea. Does it act the same?

f. Action of nitrous acid on urea. To a few c.c. of an aqueous solution of urea in a test-tube add a little dilute hydrochloric acid and one or two drops of sodium nitrite solution. An effervescence of nitrogen and carbon dioxide takes place.

Write the equation of the reaction.

g. Action of an alkali on urea. Add a little sodium hydroxide solution to some urea in a test-tube and heat to boiling. What is the reaction of the vapors?

Write the equation of the reaction.

The Cyanogen Compounds

79. Preparation of Cyanogen. Heat a small quantity of mercuric cyanide in a dry test-tube containing a cork through which projects a small, bent, glass tube. Note that white fumes are given off which condense on the cooler parts of the test-tube and tube. On igniting the gas which is given off, it will burn with a characteristic purple flame.

80. Preparation of Hydrocyanic Acid (Prussic Acid). (Demonstration). 10 c.c. of a cold saturated solution of potassium ferrocyanide and 20 c.c. of a 20 per cent solution of sulphuric acid are placed in a small distilling flask, which is immediately connected with a condenser. The receiving end of the condenser is dipped into a test-tube containing a 1/10 normal solution of sodium hydroxide. On heating hydrocyanic acid distils, which is converted into sodium cyanide. Hydrocyanic acid is so excessively poisonous that it is advisable to take these precautions. The reactions of the salts are analogous; hence, they will be used in studying the properties of this compound.

81. Reactions of Hydrocyanic Acid. *a. Prussian-blue test.* To a little of the solution obtained in Exp. 80, or to a 1 per cent solution of potassium cyanide, plus an equal volume of a 1 per cent solution of sodium hydroxide, add a little ferrous sulphate and boil for a few seconds. On acidifying this solution and adding a drop or two of ferric chloride, a precipitate of Prussian-blue is formed.

Write the equation of the reaction.

b. Hydrolysis induced by boiling. Boil about 20 c.c. of a 1 per cent solution of potassium cyanide. It is hydrolyzed into potassium formate and ammonia. Test the vapors with red litmus.

CHAPTER XI

LIPINS

The Fats. Neutral esters of glycerol and fatty acids.

82. Solubility. Test separately the solubility of lard and cottonseed oil in each of the following solvents: Water, cold alcohol, hot alcohol, ether, chloroform, and petroleum-ether.

Record your results.

83. Formation of Crystals. Dissolve about a gram of lard in a little ether in a test-tube and add an equal volume of alcohol. Pour the solution into a clean dry watch-glass; set in a quiet place; and allow the solvents to evaporate spontaneously. Examine the residue under a microscope.

Does it show crystalline formation?

84. Reaction. Dissolve a few c.c. of *fresh* cottonseed oil in a little alcohol, and then test its reaction to litmus, to Congo red, and to phenolphthalein.

Repeat the test, using *rancid* cottonseed oil.

Record your results and explain them.

85. Emulsification. Prepare 6 test-tubes as follows:

1. Ten c.c. of water and 2 c.c. of neutral cottonseed oil.
2. Ten c.c. of water, 2 drops of 5 per cent NaOH, and 2 c.c. of neutral cottonseed oil.
3. Ten c.c. of water, 2 drops of oleic acid, and 2 c.c. of neutral cottonseed oil.
4. Ten c.c. of water, 2 drops of 5 per cent NaOH, 2 drops of oleic acid, and 2 c.c. of neutral cottonseed oil.
5. Ten c.c. of water, 2 drops of 5 per cent NaOH, and 2 c.c. of rancid cottonseed oil.
6. Ten c.c. of water, 2 drops of 5 per cent NaOH, and 2 c.c. of commercial olive oil.

Shake each thoroughly for one minute; set aside; examine at the end of 5, 15, 30 and 60 minutes; record your observation; then answer the following questions:

What sort of an emulsion do you get in each case, and why?

What is an emulsion? What is a temporary emulsion? What is a permanent emulsion?

86. Reaction of Fats with Bromine. Dissolve 10 drops of olive oil in a little alcohol, add an equal volume of bromine water and shake thoroughly. Is the bromine absorbed? Explain this reaction.

87. Acrolein Test. Mix a small amount of lard with an equal amount of potassium hydrogen sulphate in a dry dish; by means of a dry stirring rod transfer a small portion of the mixture to a dry test-tube; heat cautiously, and observe the irritating odor of acrolein. Also, moisten a strip of filter paper with a drop of ammoniacal silver nitrate and hold it in the fumes of the acrolein. What happens? Why?

88. Saponification. Place 10 grams of lard in a flask, add 30 c.c. of alcoholic sodium hydroxide solution and warm on a water-bath until the saponification is complete. This point is indicated by the complete solubility of a drop of the solution when allowed to fall into a little water. Now transfer the solution to an evaporating dish containing 40 c.c. of water and heat on a water-bath until all the alcohol has been driven off. Precipitate the fatty acids with hydrochloric acid and cool the solution. Remove the fatty acids which rise to the surface. Reserve the fatty acids for future experiments. Neutralize the remaining solution with a solution of sodium carbonate, and evaporate to dryness. Extract the residue with alcohol, then evaporate this alcohol extract on a water-bath until all the alcohol is removed. This residue is mainly glycerol. Reserve it for use in the following experiments:

89. Reactions of Glycerol. *a. Solubility.* Test the solubility of glycerol in water, ether, and alcohol.

b. Acrolein test. Repeat the test as outlined in Exp. 87, using a few drops of glycerol instead of lard.

c. Borax fusion test. Mix a few drops of glycerol with some powdered borax. Dip a platinum wire in this mixture and fuse it. Note the characteristic blue flame.

d. Dunstan's test. Add phenolphthalein solution to a 5 per cent solution of borax until a permanent pink color is produced. Add an aqueous solution of glycerol (about 10 per cent), drop by drop, until the color is just discharged. Boil the solution. The color will return if excess of glycerol has not been added.

NOTE.—Any polyhydric alcohol may give this reaction. Ammonia salts also decolorize the solution, but the color does not return on heating.

e. Solution of cupric hydroxide. Add a few drops of sodium hydroxide solution to about 5 c.c. of copper sulphate solution. Cupric hydroxide is precipitated. Add a little glycerol to this suspension and note what occurs.

90. Properties of the Fatty Acids. Prove by the following tests that the precipitate obtained in Exp. 88 consists of fatty acids:

a. Reaction. Dissolve a small portion in ether and add it to about 5. c.c. of alcohol containing 1 drop of phenolphthalein solution and 1 drop of dilute sodium hydroxide solution. Is the color dispelled?

b. Acrolein test. Repeat Exp. 87, using a small portion of the fatty acids instead of the fat. What is your result? Why?

c. Formation of soap. Dissolve some of the fatty acids in sodium hydroxide solution. Is a soap lather produced on shaking some of this mixture with warm water? Add some solid sodium chloride to another portion, to the point of saturation, with continual stirring. Is soap precipitated? Explain this reaction. To a third portion add some calcium chloride solution. Do you get the usual curdy precipitate produced by calcium soaps?

Distinguish between hard soaps and soft soaps.

CHAPTER XII

THE CARBOHYDRATES AND GLUCOSIDES

General Tests on the Carbohydrates

91. Solubility of Typical Carbohydrates. Test the solubility of *arabinose, dextrose, levulose, sucrose, maltose, lactose, starch, dextrin,* and *cellulose* in the so-called "biological solvents": H_2O; 10 per cent NaCl; 0.5 per cent Na_2CO_3; 0.2 per cent HCl; 8 per cent NaOH; concentrated HCl; ether, and alcohol. Use only a pinch of the carbohydrate and about 5 c.c. of the solvent in each case. Record your results.

92. Molisch's Test (Furfuraldehyde Reaction). Place about 5 c.c. of concentrated sulphuric acid in a small test-tube. Incline the tube to an angle of 45° and slowly pour into it about 5 c.c. of a weak solution of any of the carbohydrates mentioned in Exp. 91, to which 2 drops of Molisch's reagent (a 15 per cent alcoholic solution of α-naphthol) has been added. A reddish-violet zone is produced at the point of contact. The reaction is due to the formation of furfuraldehyde by the acid, which then combines with the α-naphthol. At the junction of the two liquids a green ring may at first be formed (because of the slight nitrate or nitrite content of the sulphuric acid), but above this in a short time the reddish-violet will appear. The band disappears after a time. All the carbohydrates and substances containing a carbohydrate group give this reaction.

MONOSACCHARIDES

A. The Pentoses, $C_5H_{10}O_5$.

In the plants and more particularly in certain gums, complex carbohydrates, called pentosans, occur. These

pentosans through hydrolysis by acids may be transformed into pentoses.

93. Action of Strong Hydrochloric Acid. Pentoses on boiling with hydrochloric acid yield furfuraldehyde, which is volatile with steam and may be detected with aniline acetate.

Place about 2 grams of gum arabic in a small flask, add about 25 c.c. 10 per cent HCl solution, and boil. Moisten a piece of filter paper with a drop of anilin acetate (prepared from equal parts of aniline, glacial acetic acid, and water). Hold this paper in the vapor escaping from the flask after most of acid fumes have escaped, a bright crimson color will be formed.

94. Tollen's Phloroglucinol Test. Mix equal parts of concentrated HCl and water, and a little phloroglucinol. Then add a small quantity of pentose solution (a 1 per cent solution of gum arabic may be used), and heat the mixture on a boiling water-bath. The solution gradually becomes cherry red in color, and shows an absorption band between D and E when examined with a spectroscope. Galactose and glycuronic acid will give the same color reaction, but glycuronic acid will not give the same absorption band in the spectroscope, and galactose exhibits no absorption bands.

95. Tollen's Orcinol Test. Repeat Exp. 94, using orcinol instead of phloroglucinol. A succession of colors from red through reddish-green to green is produced. A green precipitate is formed which is soluble in amyl alcohol and has absorption bands between C and D in the spectroscope.

B. The Hexoses, $C_6H_{12}O_6$.

a. Glucose. Synonyms Destrose, grape sugar.

$$(CH_2OH)—(CHOH)_4—(CHO).$$

For the following tests use a 2 per cent solution of glucose.

96. Formation of Esters (Alcohol Reaction). Shake up a little of the glucose solution with a few drops of benzoyl chloride and an excess of sodium hydroxide solution. A precipitate is formed consisting of the benzoyl ester of glucose. Since any alcohol will yield this reaction it would be necessary to determine the melting-point of the ester in order to use this test as a means of identification; however, this may be omitted in this instance.

97. Moore's Test (Aldehyde Reaction). To about 5 c.c. of the glucose solution add an equal volume of sodium hydroxide solution, and boil. The liquid acquires a color ranging from yellow to dark brown. The intensity of the color depends on the quantity of glucose present. The brown color is due to the oxidation of the carbohydrate with formation of alkali salts of certain organic acids (lactic acid, glucic acid, etc.). This test is unreliable as a test for sugar in urine.

98. Action of Concentrated Hydrochloric Acid on Glucose. Boil a few c.c. of the glucose solution with an equal volume of conc. HCl. The solution becomes brown, and black "humic substances" separate out. Levulinic acid is formed and remains in solution. What kind of an acid is levulinic acid?

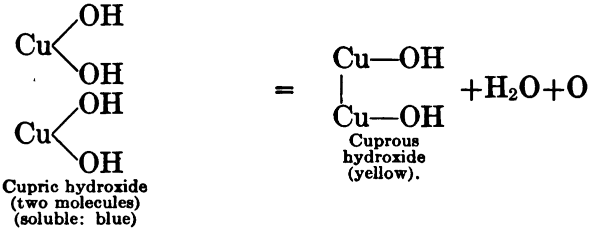
99. Reduction of Metallic Oxides in Alkaline Solutions. To their aldehyde or ketone structure many sugars possess the property of readily reducing the alkaline solutions of the oxides of metals like copper, bismuth and mercury. They also possess the property of reducing ammoniacal silver solutions with the separation of metallic silver. Most of the best-known tests for sugars are based upon this reducing property.

a. Reduction of Fehling's solution. (This solution must be prepared and kept in two separate bottles "A" and "B" and just before using they are mixed in equal proportions. "A" contains 69.28 grams of copper sulphate dissolved in water and made up to a litre. "B" contains 346 grams of Rochelle salt plus 130 grams of sodium hydroxide

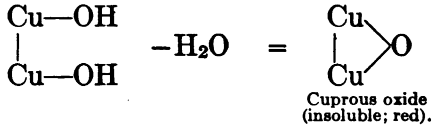
dissolved in water and made up to a litre.) The mixed Fehling's solution is of such a strength that 1 c.c. will reduce exactly 0.005 gram of glucose; hence, it may be used for quantitative tests. For qualitative tests it is desirable to dilute the solution before using. Dilute about 5 c.c. of the mixed Fehling's solution with about 10 c.c. of water and heat to boiling; if no precipitate forms (due to the decomposition of the Fehling's solution) add 1 c.c. of the dextrose solution and heat again.

The production of yellow cuprous hydroxide or brownish-red cuprous oxide indicates that reduction has taken place. These changes are indicated as follows:

The alkali converts the copper sulphate into cupric hydroxide, and this, in turn, is reduced by the carbohyhydrate. Equations:



Cu
OH
OH
Cu
OH
OH
Cupric hydroxide (two molecules) (soluble: blue)
=
Cu—OH
Cu—OH
Cuprous hydroxide (yellow).
$+H_2O+O$



b. Reduction of Fehling-Benedict's sugar reagent. (17.3 grams $CuSO_4$, 173.0 grams sodium citrate and 100.0 grams of sodium carbonate dissolved in a litre of water.) This solution can all be made up in one bottle and does not decompose on standing as does the Fehling's solution, and it is more delicate. Boil a little of this solution in a test-tube and add an equal volume of dilute sugar solution. Compare the result with Fehling's test.

c. Barfoed's sugar solution. (This solution is prepared by dissolving 4.5 grams of copper acetate in 100 c.c. of

water and adding 1.2 c.c. of 50 per cent acetic acid.) Place about 5 c.c. of Barfoed's solution in a test-tube and heat to boiling. Add dextrose solution slowly, a few drops at a time, heating after each addition. If a precipitate does not form upon continued boiling, allow the tube to stand a few minutes and examine. Barfoed's test is specific for monosaccharides.

d. Nylander's reagent (alkaline bismuth), (20 grams of bismuth subnitrate and 40 grams of Rochelle salt are dissolved in a litre of 10 per cent sodium hydroxide solution). To about 5 c.c. of the glucose solution add ½ c.c. of Nylander's reagent and heat for several minutes in a boiling water-bath. Note what happens. Explain.

e. Reduction of silver ammonium hydroxide. Place about 5 c.c. of silver nitrate in a perfectly clean test-tube; then add ammonium hydroxide, drop by drop, until the precipitate which first forms just redissolves. Add a few drops of the glucose solution and heat in a boiling water-bath without agitation for a few minutes. A mirror of metallic silver gradually forms.

Write the equations of the above reactions.

100. Phenylhydrazine Reaction (Osazone Test). To about 5 c.c. of the glucose solution in a clean test-tube add about 2 grams of the phenylhydrazine mixture (1 part by weight of phenylhydrazine-hydrochloride and 2 parts by weight of sodium acetate, thoroughly mixed in a mortar), shake well and heat on a boiling water-bath for half an hour, and allow the mixture to cool gradually. Examine the deposit under the microscope and sketch the crystals. Certain other carbohydrates respond to this test but the crystals have different configurations.

Write the reactions (consult your text-book).

101. Fermentation Test. Rub up in an evaporating dish a small piece of yeast (¼ of a yeast cake), with about 25 c.c. of glucose solution. Place the mixture in a saccharimeter and let it stand in a warm place until the next day. Note the result and explain it. What is the gas that

has collected in the upper end of the tube? Prove the presence of alcohol in the liquid by applying the iodoform test. (Exp. 22, *c*.)

102. Determination of the Specific Rotation. The property possessed by all carbohydrates of rotating the plane of polarized light in direct proportion to the amount contained in the solution under examination is utilized for the purpose of determining the percentage of carbohydrate that is present. Some substances, such as glucose, rotate the plane of polarization in the direction of the hands of a watch. These are called dextrorotatory and are designated with a + sign. Substances such as fructose which rotate the plane of polarization in the opposite direction are called lævorotatory and are designated with a − sign.

The "specific rotating power" of a substance is the angle about which the plane of polarized light is turned by a layer 1 cm. thick of a solution containing in 1 c.c. 1 gram of the optically active substance. It is dependent upon the wave length of the light and upon the temperature; hence, it is necessary to specify both of these in stating the value of the specific rotation. With most substances it is impractical to estimate the specific rotation of a 100 per cent solution, but it has been calculated from the rotations of solutions of known strengths. The symbol $(\alpha)_D$ is used to express this value, the *D* standing for sodium light.

The following are the values for a few of the principal carbohydrates in 10 per cent solutions estimated at 20° C.:

Glucose = +52.8°
Galactose = +83.0
Fructose = −93°

Lactose = + 52.5°
Maltose = +138°
Sucrose = + 66.5°

Starch = +199°
Glycogen = +196.6°

The formula is as follows:

$$(\alpha)\frac{20^\circ}{D} = \frac{\alpha \times 100}{C \times l}$$

In which $(\alpha)\frac{20^\circ}{D}$ = specific rotation;
α = observed rotation;
c = concentration;
l = length of tube in decimeters.

The use of the polariscope. The instrument used for the purpose of estimating the amount of an optically active substance is called a polariscope, or polarimeter, and essentially consists of a polarizer and an analyzer, the latter being used to determine the direction and degree of rotation which the light has undergone when passing through the polarizer. The instrument is so arranged that when the analyzer is in one position, one half of the field is fully illuminated while the other half is dark, the two portions being sharply divided by a vertical line. On turning the analyzer to the opposite extreme, the light and the dark portions are transposed. The polariscope is so constructed that a glass tube filled with the liquid to be examined can be placed horizontally in the track of the polarized ray, between the polarizing and the analyzing sections. Before the tube which contains the solution to be analyzed is placed in position, the zero point of the vernier attached to the eyepiece is made to coincide with the zero point of the circular scale of degrees from which the reading is taken. At this point the whole field should be equally illuminated. If it is not, the position of the prisms must be regulated by means of an adjusting screw provided for the purpose, until perfect equality is obtained. The tube is now placed in position and the screw is turned until the two halves of the field are equally illuminated. A reading is then taken by means of the vernier. Read off the number of whole degrees, half degrees, and minutes. Take several readings by moving the lever and coming back to the point where the two halves of the field are equal.

The resulting value gives you alpha. Knowing alpha the concentration of the carbohydrate solution and the

length of the tube, the specific rotatory power may be calculated from the formula given above.

The determination of glucose by means of the polariscope. The amount of glucose in a solution may be calculated from the following equation:

$$X=\frac{\alpha\times100}{52.8\times1}$$

The tubes are usually either 100 or 200 mm. in length, but by using a tube of such a length as to render the degrees or arc equal to percentages, the necessity for calculation is obviated. For instance, in estimating glucose, if a tube 189.4 mm. long be used, then the equation becomes

$$X=\alpha$$

and the amount of glucose in 100 c.c. of the solution becomes the observed angle of rotation. If a tube 94.7 mm. in length be used, then the amount of glucose in 100 c.c. is double the observed angle.

There are many types of special polariscopes on the market which are made solely for the purpose of estimating the percentage of sugar in solution. In these instruments the degree of rotation produced by the sugar solution is ascertained by means of a quartz wedge which, when moved by a screw, compensates the rotation; the extent to which the wedge has to be moved to produce compensation is indicated in percentages on the horizontal scale which takes the place of the degrees of arc of the ordinary polariscope. With these special polariscopes, usually called saccharimeters, any bright artificial light may be used, whereas with the other polariscopes, monochromatic (usually sodium) light is used.

b. Fructose. Synonyms: Levulose, fruit sugar.

$$(CH_2OH)—(CHOH)_3—C=O—(CH_2OH).$$

103. Properties of Fructose. Repeat Exps. 96 to 102. Do you obtain the same results as with glucose?

NOTE.—(*a*) Although fructose is a ketose, it, nevertheless, reduces metallic oxides in alkaline solution. This is due to the terminal, CO—CH_2OH, group, which is easily oxidizable. Though acetone does not produce metallic oxide, monohydroxy acetone CH_3—CO—CH_2OH does, as it contains the —CO—CH_2OH group.

(*b*) Fructose gives the same osazone as dextrose. Compare the crystals under the microscope. Write the equations to show why the end product in each case is the same.

104. Seliwanoff's Special Test for Fructose. To a mixture of equal parts of concentrated hydrochloric acid and water add a very small quantity of fructose solution and then add a few crystals of resorcinol. Heat. The solution becomes red in color and deposits a brownish-red precipitate which dissolves in alcohol, giving a red solution.

c. Galactose, $CH_2OH(CHOH)_4$—CHO.

Galactose occurs with dextrose as one of the products of hydrolysis of lactose. In general the same reactions are given by galactose as by glucose. If differs from glucose in having a higher dextrorotatory power and in forming a different osazone.

105. Properties of Galactose. Repeat Exps. 94, 99 and 100, using a 1 per cent solution of galactose instead of glucose. Record your results.

106. Mucic Acid Test. Place 50 c.c. of the galactose solution in a beaker; add 15 c.c. of concentrated nitric acid and evaporate the mixture on a boiling water-bath until the volume has been reduced to about 15 c.c. At this point the liquid should be clear, and a fine white precipitate of mucic acid should form.

DISACCHARIDES. $C_{12}H_{22}O_{11}$

a. Sucrose. Synonyms: Saccharose, sugar, cane sugar, and beet sugar.

A 2 per cent aqueous solution of pure crystalline sucrose will be furnished.

107. Properties of Sucrose. Repeat Exps. 96, 97, 98, 99, 101, 102 and 104. Record your results and compare with the corresponding reactions obtained with glucose and levuiose.

108. Hydrolysis of a Disaccharide. To 10 c.c. of the sucrose solution add about 2 c.c. of 10 per cent H_2SO_4 solution and boil for a few minutes. Cool, render alkaline with solid NaOH, and then perform the reduction test. Use the Fehling-Benedict reagent. (Exp. 99, *b.*)

Explain the result after comparing with your result obtained with the reduction test with sucrose in Exp. 107.

b. Maltose, or malt sugar

109. Properties of Maltose. With a 2 per cent aqueous solution of maltose perform Exps. 97, 98, 99, 100, 101, and 104. Compare with the corresponding reactions with glucose, levulose, and sucrose. Make a drawing of the asozone crystals.

c. Lactose, or milk sugar

110. Properties of Lactose. With a 2 per cent solution of lactose perform Exps. 97, 98, 99, 100, 101, 104, and 106, as described under the monosaccharides. Record your results and compare with the corresponding reactions of the preceding carbohydrates.

POLYSACCHARIDES

a. Starch. Synonym: Amylum. $(C_6H_{10}O_5)_n$

111. Preparation of Starch from Potato. Scrape a medium-sized potato as finely as possible with a knife or

spatula, collect the scrapings in a casserole, add about 100 c.c. of water, stir thoroughly, and strain through a piece of unbleached muslin. Mix the residue in the beaker with more water, allow the starch to settle, and decant off the supernatant liquid. Repeat this washing and decanting until a pure white residue remains in the bottom of the beaker. Allow the compact mass of starch to drain thoroughly and spread it out to dry. This preparation may be used in the following experiments:

112. Preparation of Starch Paste. Place 200 c.c. of water in a casserole and heat to boiling. In a small beaker mix 2 grams of starch with a few c.c. of cold water and pour the mixture into the boiling water with constant stirring. Boil the starch paste for a few seconds.

Cool, by placing the casserole in a pan of cold water.

113. Action of Alcohol on Starch Paste. Place 10 c.c. of the dilute starch paste in a test-tube and gradually add 95 per cent alcohol until no further action is observed.

114. Action of Iodine on Starch Paste. Place 2–3 c.c. of starch paste in a test-tube and add a drop of iodine solution (1/10 normal iodine solution = 13 grams of iodine dissolved in 30 grams of potassium iodide in 250 c.c. of water and made up to a liter), and observe the production of a deep blue color. Heat the tube and note what happens. Allow the tube to cool and again note what happens. How do you explain these phenomena? Could these color changes be obtained in an alkaline solution? Why?

115. Action of Iodine on Starch Granules. Cut a *very* thin slice of potato, or take a few starch granules, and place on a slide and examine under a microscope. Now put a small drop of iodine solution on the slice of potato and again examine under the microscope. What do you conclude from this experiment in regard to the nature of starch granules.

116. Action of Tannic Acid on Starch Paste. To a small amount of starch paste in a test-tube add an equal volume of a 1 per cent solution of tannic acid. The solution should

become strongly opaque and ordinarily a yellowish-white precipitate is formed.

117. Action of Basic Lead Acetate on Starch Paste. To a few c.c. of starch paste add an equal volume of basic lead acetate solution and note what happens.

118. Diffusibility of Starch Paste. Test the diffusibility of starch paste through animal membrane, parchment paper or collodion (see model).

119. Action of Fehling's Solution on Starch Paste. On mixing equal volumes of starch paste and Fehling's solution and heating to boiling, do you get a reduction?

120. Hydrolysis of Starch. Place about 20 c.c. of starch paste in a small beaker, add 10 drops of concentrated hydrochloric acid, and boil. By means of a small pipette at the end of each minute remove a drop of the solution and place it on a porcelain test tablet, or crucible cover, and make the iodine test (see Exp. 114). As the hydrolysis proceeds, the blue color should gradually fade and finally disappear. At this point, cool a few c.c. rapidly in a test-tube and add an excess of alcohol.

If this solution does not become opaque on addition of alcohol (thereby indicating that all the starch has been hydrolyzed), cool the remainder of the solution and render a portion of it alkaline with solid NaOH and perform the Fehling's test. A positive Fehling's test indicates that a reducing sugar has been formed from the starch. Make a phenylhydrazine test (Exp. 100), on another portion of the hydrolyzed starch. What sugar has been formed?

121. Microscopical Examination of Starch Granules. Examine microscopically and make a sketch of the configuration of the granules of each of the following starches: Potato, corn, arrowroot, wheat, and rice.

b. Dextrin

122. Properties of Dextrin. Repeat Exps. 113, 115, 116, 117, 118, 119 and 120, using a 1 per cent solution of dextrin instead of starch paste. Record your results.

NOTE.—Commercial dextrin usually contains more or less unaltered starch, also more or less reducing sugar. In the former case the iodin may give a blue or violet color (a mixture of blue and red). In the latter case reduction takes place on testing with Fehling's reagent. Pure dextrin gives no coloration with Fehling's reagent.

123. Testing Bread Crust for Dextrin. Test bread crust for dextrin and compare with a portion from the center of the loaf. Record your results.

c. Glycogen

124. Preparation of Glycogen from Scallops. Place a few scallops in a mortar, add a half teaspoonful of sand, and grind the scallops into a pulp, thereby rupturing the cells. Transfer to a casserole, add about 50 c.c. of water, and boil for 20 minutes. Note the opalescence of the solution. At the boiling-point faintly acidify with acetic acid. Why? Filter, and use the solution for the following tests:

125. Iodine Test. To about 5 c.c. of the glycogen add a drop or two of a 10 per cent solution of sodium chloride (the NaCl sol. is not absolutely necessary, but it intensifies the reaction). Then add 2 to 3 drops of iodine solution. Note carefully the color formed. How does it compare with the colors produced by iodine on starch or dextrin?

126. Action of Fehling's Solution on Glycogen. Make a portion of the glycogen solution alkaline. Use solid NaOH. Then perform the Fehling's test. Do you get a positive result?

127. Hydrolysis of Glycogen. Add 10 drops of concentrated hydrochloric acid to 10 c.c. of the glycogen solution, and boil for 10 minutes. Cool. Make alkaline with solid NaOH, and test with Fehling's solution. Now, what is your result?

128. Action of Alcohol, Tannic Acid, and Basic Lead Acetate on Glycogen. Repeat Exps. 113, 115 and 116 on small portions of the glycogen solution, and compare with results obtained on starch and dextrin.

d. Cellulose

129. Solubility of Cellulose. Recall your tests on the solubility of cellulose as noted in Exp. 91. In addition try its solubility in the following "special solvents": *a.* Schweitzer's reagent. (This reagent may be prepared by adding sodium hydroxide solution to a solution of copper sulphate containing some ammonium chloride. A precipitate of copper hydroxide is formed, filtered off, and washed with water. The moist copper hydroxide is added to concentrated ammonium hydroxide until no more dissolves.) When placed in this solution, filter paper (cellulose) dissolves. On addition of dilute hydrochloric acid, the color is discharged and an amorphous flocculent precipitate of cellulose is deposited.

b. Stannous chloride in aqueous solution, or better, stannous chloride in HCl solution.

c. Mercuric chloride in HCl solution.

130. Formation of Parchment Paper. Into a cold mixture of two volumes of concentrated sulphuric acid and one of water, dip a piece of dry filter paper for a few seconds; remove and wash it at once in water. This treatment converts the filter paper into amyloid (parchment paper), which gives a blue coloration with iodine. Even while wet, parchment paper is very tough as compared with the original dry filter paper.

GLUCOSIDES

Whereas most carbohydrates exist in nature in the free state, there also occur a large number of complex compounds, which on hydrolysis are broken down into a mono- or disaccharide, and one or more other compounds. The hydrolysis is produced both by boiling with acids and by treating with enzymes at body temperature. The sugar is, in most cases, glucose, but certain glucosides yield some other variety of sugar. The substances combined with the sugar may or may not have a physiological action.

131. Reactions of Salicin. *a.* Ascertain if salicin will reduce Fehling's solution. *b.* Hydrolyze about 10 c.c. of a 2 per cent aqueous solution of salicin by adding 15 to 20 drops of dilute sulphuric acid and boiling for about 15 minutes. Now, make alkaline with sodium hydroxide solution and again test for reducing sugar with Fehling's reagent.

132. Tests for Digitalin. The great toxicity of such a glucoside as digitalin makes it advisable to include tests for its detection. Autenrieth advises the following tests:

a. Concentrated sulphuric acid colors pure digitalin orange yellow. This solution soon becomes blood red, changing upon adding a drop of nitric acid or ferric chloride to cherry and blue red.

b. Concentrated hydrochloric acid dissolves it with a golden yellow color, changing with heat to garnet or violet-red.

Only traces of digitalin are necessary for these tests.

133. Scheme for the Identification of the Most Important Carbohydrates. 1. Apply the Molisch test to a few c.c. of the solution. (Exp. 92.) A positive test indicates that some carbohydrate or substance containing a carbohydrate group is present. A negative result by this reaction is very good evidence of the absence of carbohydrates. Consequently, if a negative result is obtained, no further tests need be applied.

2. Test the reaction of the solution to litmus paper. If the solution is alkaline, neutralize or make faintly acid with HCl. Test a few drops of the faintly acid solution with iodine solution. A blue color indicates *starch.* A reddish color indicates *erythrodextrin* or *glycogen.* Solutions of *dextrins* are transparent and the dextrins *are not* precipitated from solution by basic lead acetate. Solutions of *glcyogen* are opalescent and glycogen *is* precipitated from solution by basic lead acetate.

3. Apply Fehling's test (Exp. 99, *a* or *b*) to about 5 c.c. of the original "unknown" solution. Reduction indicates *dextrose, levulose, galactose, pentose, maltose,* or *lactose.*

4. Test another portion of the "unknown" solution with Barfoed's reagent (Exp. 99, *c*). A positive test indicates the presence of *dextrose, levulose, galactose* or *pentose.* A negative Barfoed's test and positive Fehling's test indicates *lactose* or *maltose.*

5. Differentiate between *lactose* and *maltose* by applying the phenylhydrazine reaction (Exp. 100), and the fermentation test (Exp. 101), to small portions of the "unknown" solution. Distinctive osazone crystals are yielded by both lactose and maltose. Of the two only maltose is fermentable with the yeast ferments.

6. To identify *levulose, dextrose, galactose* and *pentose* proceed as follows: Test by Seliwanoff's reaction (Exp. 104). A positive test indicates levulose. Perform the phenylhydrazine reaction (Exp. 102). Dextrose and levulose yield the same osazone crystals, but dextrose yields a negative Seliwanoff's reaction; hence, a negative Seliwanoff's reaction and positive glucosazone crystals indicate dextrose. Galactose and the pentoses likewise yield distinctive osazone crystals. *Galactose* may also be identified by boiling some of the "unknown" solution with nitric acid. A fine white precipitate of mucic acid is obtained. To further identify the *pentoses* apply the phloroglucinol test (Exp. 94), and the orcinol test (Exp. 95).

7. To identify *sucrose* in the absence of reducing sugars proceed as follows: Add 1 drop of concentrated HCl to about 5 c.c. of the "unknown" solution and boil for one minute. Cool, render alkaline with NaOH solution and test by Fehling's reaction. Reduction indicates the presence of sucrose.

8. *Sucrose* may be detected in the presence of other disaccharides and polysaccharides as follows: Apply Barfoed's test to insure the absence of monosaccharides in the "unknown" solution (levulose is the only one that must be absent); then test by Seliwanoff's reaction. A positive test indicates *sucrose.* Why?

CHAPTER XIII

MONOBASIC UNSATURATED ACIDS, SATURATED DIBASIC ACIDS, AND HYDROXY ACIDS

134. Absorption of Halogens by Unsaturated Acids. Dissolve a few drops of oleic acid in a little carbon tetrachloride, and add to this a few drops of a solution of bromine in carbon tetrachloride. Note what happens. Explain.

135. Elaidic Transformation. Pour about 5 c.c. of oleic acid into a test-tube and add a small piece of sodium nitrite ($\frac{1}{2}$ gram), and two drops of concentrated nitric acid. Allow the mixture to stand a few minutes. Owing to a shifting of the double bonds, the liquid oleic acid is transformed into its solid isomer, *elaidic acid.*

136. Preparation of Oxalic Acid. (Demonstration.) 180 c.c. of concentrated nitric acid are placed in a large flask (2 liters), and the acid is warmed on a water-bath. The flask is then placed in a hood and 50 grams of sucrose added. Torrents of brown fumes are evolved. When this reaction ceases, the liquid is evaporated to about $\frac{1}{4}$ its original volume. On cooling, large, colorless, prismatic crystals of oxalic acid separate.

137. Reactions of Oxalic Acid. *a.* Heat a little oxalic acid on a crucible lid, and note that it volatilizes without charring.

b. Mix about 2 grams of oxalic acid and 2 c.c. of concentrated sulphuric acid in a test-tube containing a cork, a small, bent, glass tube. Note that effervescence ensues, without charring, and that the gas which is evolved may be ignited. What gas is it that is inflammable?

Write the equation of the reaction.

c. Oxidation of oxalic acid with potassium permanganate. Acidify a little oxalic acid solution with sulphuric acid and

warm gently. Add some $KMnO_4$ solution, drop by drop, with constant stirring. It is at first decolorized, but when the oxalic acid is all oxidized the pink color remains. Write the equation of the reaction.

d. Salts of the alkaline earths. To a little dilute ammonium oxalate solution add a few c.c. of $CaCl_2$ or $BaCl_2$ solution. Note the heavy precipitate that is formed in either case. Test the solubility of calcium and barium oxalate in water, in dilute acetic acid and dilute hydrochloric acid.

138. Detection of Lactic Acid ($CH_3CHOH—CO—OH$). *a. Action of very dilute ferric chloride.* To about 5 c.c. of a very dilute solution of ferric chloride (nearly colorless), add a few drops of a dilute solution of lactic acid, or "unknown" solution. The solution becomes deeply yellow.

b. Uffelmann's test. To a 2 per cent aqueous solution of phenol add, drop by drop, an aqueous solution of ferric chloride until an amethyst blue color is obtained. On adding a little dilute lactic acid solution to a few c.c. of Uffelmann's reagent the amethyst-blue color of the reagent is displaced by a straw yellow.

NOTE.—Mineral acids such as hydrochloric acid discharge the blue color, leaving a colorless solution; other organic acids also give a yellow or brownish color. To detect lactic acid in the presence of a mineral acid it is necessary to shake the solution with a few c.c. of ether; to remove the ether by means of a separatory funnel; to evaporate the ether to dryness; to take up the residue in water, and then to perform the test.

139. Reactions of Tartaric Acid.

$$HO—CO—(CHOH)_2—OC—OH.$$

1. Heat a little tartaric acid in a test-tube. Does it char? What does the odor resemble?

2. Are the alkali salts of tartaric acid very soluble? Add a little KNO_3 solution and a few drops of dilute acetic acid to a strong solution of tartaric acid and stir with a glass rod. The acid potassium salt of tartaric acid is precipitated. A similar precipitate is formed when an ammonium salt

is used in place of the potassium salt. Test the solubility of your hydrogen potassium tartrate in water, dilute acetic and dilute hydrochloric acids.

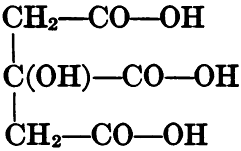
3. Tartaric acid prevents the precipitation of metallic hydroxides by NaOH.

a. To a little ferric chloride solution add NaOH and then some sodium potassium tartrate solution. A reddish-brown precipitate of ferric hydroxide is found, which dissolves on adding the tartaric and gives a yellowish-brown solution.

b. Repeat the same reaction, using $CuSO_4$ solution instead of the ferric chloride. What do you observe? This property is used in the preparation of Fehling's solution.

140. Preparation of Citric Acid from Lemons.

Citric acid.



$$\begin{array}{l} CH_2—CO—OH \\ | \\ C(OH)—CO—OH \\ | \\ CH_2—CO—OH \end{array}$$

Express the juice from half a lemon, add to it an equal volume of water. Filter the mixture and make alkaline with ammonium hydroxide. Add 5 c.c. of a 5 per cent solution of calcium chloride and heat to boiling. What is the formula of the compound formed? Allow the liquid to cool. Does the precipitate remain?

CHAPTER XIV

CARBOCYLCIC COMPOUNDS

A. BENZENE AND ITS HOMOLOGUES

141. Preparation of Benzene, C_6H_6. Mix thoroughly in a mortar 15 grams of benzoic acid and 50 grams of soda lime. Transfer the mixture to a dry round-bottom flask; connect with a condenser, and heat on a wire gauze until no more liquid comes over. To prevent loss by evaporation, surround the receiver with cold water.

Equation:

$$2C_6H_5COOH+Ca(OH)=(C_6H_5-COO)_2:Ca+H_2O$$

$$(C_6H_5-COO)_2:Ca+Ca(OH)_2=2C_6H_6+2CaCO_3.$$

Draw off the oily benzene with a pipette. Shake it with a little dilute sodium hydroxide solution. Recover the benzene. Add to it a little fused calcium chloride. After this mixture has stood for about an hour, decant off the liquid into a large test-tube, connect this tube to a condenser and redistil. Notice the odor of the distillate, which should be preserved for future experiments. A small test-tube fitted with a cork is suitable for this purpose.

142. Reactions of Benzene. *a. Inflammability.* Show that benzene is inflammable by pouring a small quantity of the final distillate obtained in Exp. 141 on a little water in an evaporating dish and igniting it.

b. Action of nitric acid. Treat 2 c.c. of benzene in a flask with a mixture of 4 c.c. of concentrated sulphuric acid and 2 c.c. of concentrated nitric acid. Add the acid mixture, drop by drop, and immerse the flask in cold water after each addition, in order to keep the temperature below 50°. As soon as all the acid has been added, pour the mixture

into water. An oily liquid, nitrobenzene, $C_6H_5—NO_2$, separates and sinks to the bottom. Notice the color and odor of the precipitate. Do not inhale the vapor of this substance. Empty the flask containing it into the jar in the hood.

Write the equation of the reaction.

c. Action of fuming sulphuric acid. Place 5 c.c. of fuming sulphuric acid in a flask, then add to it, drop by drop, 2 c.c. of benzene. Shake the flask, and heat it gently until the benzene, which at first floats upon the surface of the acid, has entirely dissolved. Cool, and divide into two parts. Pour part one into a test-tube two-thirds full of cold water. Does any insoluble substance separate? Pour part two into a small flask containing 10 c.c. of a saturated solution of sodium chloride, and heat this mixture until hydrochloric acid gas is evolved. On cooling, sodium benzene-sulphonate, $C_6H_5—SO_2—ONa$, separates in crystalline plates. Filter on a Büchner funnel; wash the crystals with a little cold water; dry them between filter papers; and preserve them for a later experiment.

d. Apply von Baeyer's test for double bonds to benzene. Agitate about 5 c.c. of benzene with 1 c.c. of 1 per cent solution of potassium permanganate, and 1 c.c. of a 5 per cent solution of sodium carbonate. Does the violet-red color remain unchanged? Compare with the experiments performed on the ethylene and olefine hydrocarbons. (Exp. 15, *c* and *f;* 16, *a.*)

e. Action of bromine on benzene. Place 5 c.c. of benzene and 1 c.c. of a 1 per cent solution of bromine in carbon-tetrachloride in a test-tube and shake for half a minute. Is the bromine dissolved by the benzene? Is the color discharged? Compare with ethylene, amylene and acetylene. Exp. 15, *b* and *e;* and 16, *a.*

143. Oxidation of Side-chains. Mix 2 c.c. of toluene and 5 c.c. of dilute nitric acid in a test-tube and heat. Does any reaction take place? What is the compound formed?

B. PHENOLS

144. Preparation of Phenol, C_6H_5—OH. *a. Phenol from sodium benzene sulphonate.* Weigh the crystals of sodium benzene sulphonate prepared in Exp. 142, *c*. Then fuse double this weight of sodium hydroxide in a porcelain crucible; add the sodium benzene sulphonate crystals, and continue to heat for 3 or 4 minutes. Avoid charring. Cool. Dissolve in a little water and acidify with hydrochloric acid. Then filter. Observe the odor of the filtrate and preserve it for later tests.

b. Phenol from salicylic acid. Mix intimately in a mortar 15 grams of salicylic acid and 50 grams of soda-lime. Transfer the mixture to a dry, round-bottom flask; connect with a condenser and heat on a sand-bath until no more liquid will come over. Collect the distillate in an Erlenmeyer flask surrounded by cold water. Preserve the distillate. Write all equations.

145. Reactions of Phenol. *a. Action of ferric chloride.* Add two drops of ferric chloride solution to 2 c.c. of a 2 per cent aqueous solution of phenol. Note that there is a blue-violet coloration, which disappears on adding mineral acids (HCl).

b. Action of bromine-water. To 5 c.c. of a 2 per cent aqueous solution of phenol add bromine-water until a permanent yellow color is attained. There is first a cloudiness due to the formation of mono- or di-bromophenol. These compounds are characterized by having a very penetrating smell. The further addition of bromine-water produces a precipitate of tribomophenol in the form of yellowish-white needles or flakes.

c. Action of Millon's reagent. To a few c.c. of a dilute aqueous solution of phenol add a few drops of Millon's reagent (mercurous and mercuric nitrate) and warm. Note the change in color.

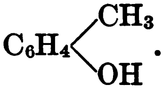
d. Action of nitric acid. Place 10 c.c. of concentrated nitric acid in a porcelain dish and add to it, drop by drop, with constant stirring, about 1 c.c. of an aqueous solution

of phenol. Heat to boiling. On cooling, picric acid, $C_6H_2(NO_2)_3—OH$, is precipitated. Write the equation. Pour the acid mixture into cold water. Examine the crystals that are formed under the microscope. Draw a picture of their configuration.

e. Apply the bromine test to the filtrate obtained in Exp. 144, *a.*

f. Apply reactions *a, b, c,* and *d* to the filtrate obtained in Exp. 144, *b.*

146. Properties of Cresols,



$C_6H_4\langle^{CH_3}_{OH}$. The three cresols, *o*-cresol, *m*-cresol and *p*-cresol, have different physical properties, such as different melting-points, but they have similar chemical properties, and they are extensively sold as a mixture of all three under the name of *tricresol.*

With a 2 per cent solution of tricresol, repeat Exp. 145, *a, b, c* and *d.* Record your results. Do they react similarly to the phenols?

C. AROMATIC AMINES AND THEIR DERIVATIVES

147. Preparation of Aniline from Nitrobenzene. Pour 5 drops of nitrobenzene into a test-tube; add about 1 gram of stannous chloride and 5 c.c. of 8 per cent sodium hydroxide solution. Warm the mixture for a minute or two. The odor of nitrobenzene disappears, giving place to that of aniline. Shake the solution with ether; pour off the ether layer into an evaporating dish; evaporate the ether very carefully by placing the dish on a water-bath containing hot water, but having no flame under it.

148. Bleaching-powder Test for Aniline. Treat the residue obtained in the last experiment with a few c.c. of a clear solution of bleaching powder; a purple-violet color appears which soon becomes dirty red.

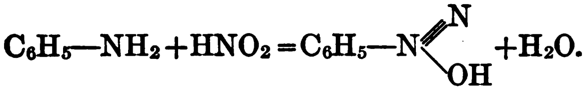
149. Formation of Tribromaniline. To an aqueous solution of aniline or one of its salts, add a little bromine water.

Note the nature of the precipitate.

150. Reaction of Aniline. Shake a few drops of aniline with about 5 c.c. of water; then test its reaction with litmus and with phenolphthalein. What are your results? How does the reaction of aniline compare with reaction of the aliphatic amines? How could you prove aniline to be a primary amine?

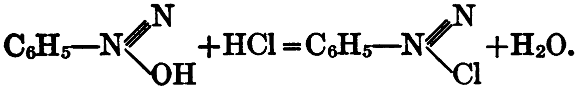
151. Preparation of Acetanilide, $C_6H_5{-}NH{-}OC{-}CH_3$. Put 3 c.c. of aniline and 5 c.c. of glacial acetic acid in a dry flask and boil under a reflux condenser for an hour and a half. Pour the mixture into water. Acetanilide is precipitated. Recrystallize it from hot water; dry between filter papers; and then determine the melting-point.

152. Action of Nitrous Acid on Aniline. Dissolve 2 c.c. of aniline in 5 c.c. of concentrated hydrochloric acid and 10 c.c. of water. Cool by placing the flask in a pan of ice water. Add 10 c.c. of a cold 10 per cent solution of sodium nitrite. The following reactions take place:



$$C_6H_5{-}NH_2 + HNO_2 = C_6H_5{-}N\begin{matrix}\equiv N\\ {-}OH\end{matrix} + H_2O.$$

(Diazobenzene)



$$C_6H_5{-}N\begin{matrix}\equiv N\\ {-}OH\end{matrix} + HCl = C_6H_5{-}N\begin{matrix}\equiv N\\ {-}Cl\end{matrix} + H_2O.$$

(Diazobenzene chloride)

Gently heat the solution containing the diazobenzene chloride. A gas is given off. What is it? Note the odor of the solution. What compound is now present in the solution? Write the equation of the reaction that took place on heating the diazonium salt.

153. Preparation of Phenylhydrazine Hydrochloride, $C_6H_5NH{-}NH_2{-}HCl$. Into 100 c.c. of concentrated hydrochloric acid in a beaker, pour slowly, with stirring, 10 c.c. of aniline. Cool the beaker to 0° by placing it in a freezing mixture of one part salt and two parts ice. Add, a few drops at a time, a 20 per cent sodium nitrite solution, until free nitrous acid can be shown to be present. A drop of the

solution diluted with a few drops of water should produce a blue color with starch-iodide paper. Stir constantly while adding the sodium nitrite solution. Dissolve 60 grams of stannous chloride in 50 c.c. of concentrated hydrochloric acid; cool it; and add it slowly to the diazo compound. After half an hour, filter off the phenylhydrazine hydrochloride on a Büchner funnel. Dry between filter papers. Write the equation of the reaction.

154. Properties of Phenylhydrazine. *a. Reduction of Fehling's solution.* Add a small quantity of the phenylhydrazine-hydrochloride to a little mixed Fehling's solution. Does reduction take place?

b. Formation of osazones. Mix a small quantity of the phenylhydrazine-hydrochloride prepared in Exp. 153 with about twice the quantity of sodium acetate, and then add to it a little glucose solution and warm on a water-bath for half an hour. Recall Exp. 100.

D. AROMATIC ACIDS

155. Properties of Benzoic Acid, $C_6H_5—CO—OH$. *a. Solubility.* Test the solubility of benzoic acid by adding about 0.1 of a gram to 5 c.c. of each of the following solvents: Cold water; hot water; alcohol; ether; dilute sodium hydroxide; and dilute hydrochloric acid. Does the benzoic acid crystallize out from the hot water on cooling? On neutralizing the sodium hydroxide solution with hydrochloric acid, is the benzoic acid precipitated?

b. Sublimation. Heat a little benzoic acid in a casserole. Note the suffocating fumes. Invert a funnel over the casserole. Observe the condensation. Examine the crystals under the microscope.

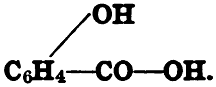
c. Action of nitric acid. To a small quantity of benzoic acid in an evaporating dish add a little nitric acid and evaporate. Observe the odor of the compound that is formed. What is it? Write the equation of the reaction.

d. Action of Millon's reagent. Heat about 0.1 of a gram

of benzoic acid with 2 c.c. of Millon's reagent. Does it yield a color reaction?

e. Action of bromine water. Add about 0.1 of a gram of benzoic acid to a little bromine water. Is the color discharged?

f. Action of ferric chloride. Add about 0.1 of a gram of benzoic acid to a little ferric chloride solution. Is there any change of color?



156. Properties of Salicylic Acid, $\overset{\diagup OH}{C_6H_4}—CO—OH$. Repeat the experiments performed on salicylic acid. Make careful note of which tests are positive. Are any of the reactions due to the presence of the —OH radical on the benzene nucleus? Compare Exps. 145 and 146.

E. AROMATIC ALDEHYDES

157. Properties of Benzaldehyde, $C_6H_5—CHO$. *a. Production of a silver mirror.* Into a perfectly clean test-tube place 5 c.c. of ammoniacal silver nitrate and add 2 or 3 drops of benzaldehyde. Immerse the tube in a water-bath containing cold water and slowly heat to boiling. Is a silver mirror produced? Compare with the aliphatic aldehydes. (Exp. 35.)

b. Action of Fehling's solution. Shake up a few drops of benzaldehyde with 10 c.c. of water, add 5 c.c. of mixed Fehling's solution and warm. Do you obtain a reduction? Compare with the aliphatic aldehydes. (Exp. 36.)

c. Spontaneous oxidation of benzaldehyde. Spread a few drops of benzaldehyde on a watch-glass and leave exposed to the air for a few days. What change has taken place?

d. Formation of addition products. Shake up 1 c.c. of benzaldehyde with 5 c.c. of a saturated solution of sodium hydrogen sulphite. Collect on a filter paper the crystalline deposit that is formed; wash it twice with a little alcohol; and dry between filter papers. Note that the odor of benzaldehyde has disappeared. Mix some of the crystals with

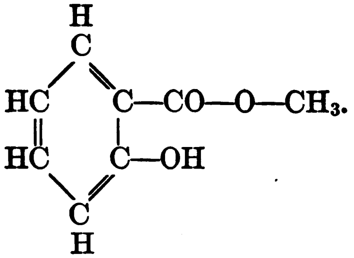
a little sodium carbonate solution and warm. Explain what happens.

e. Action of sodium hydroxide. Mix 1 c.c. of benzaldehyde with 5 c.c. of 8 per cent sodium hydroxide solution and warm. Is aldehyde resin formed? Compare with the results obtained with acetaldehyde. (Exp. 37.)

f. Action of very concentrated alkali. Place in a small flask 10 c.c. of benzaldehyde and 30 c.c. of a 60 per cent solution of potassium hydroxide. Stopper the flask with a cork and shake it until an emulsion is formed, and set it aside until the next day. Add sufficient water to dissolve the crystals of potassium benzoate which have formed. Transfer the mixture to a separatory funnel and extract twice with 25 c.c. portions of ether. Remove the traces of water that remain in the ethereal solution by shaking with anhydrous copper sulphate. Distil off the ether. And finally, distil the benzyl alcohol, $C_6H_5—CH_2OH$, which has formed. It should boil at 206°.

F. POLYSUBSTITUTION PRODUCTS OF BENZENE

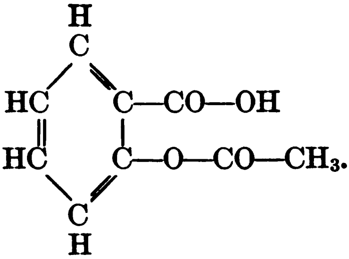
158. Preparation of Methyl Salicylate (Oil of Wintergreen),



Mix together on a watch-glass about 0.1 of a gram of salicylic acid, 5 drops of methyl alcohol, and 5 drops of concentrated sulphuric acid, and gently warm. Observe the odor of the compound formed.

159. Preparation of Aspirin. Heat 5 grams of finely powdered salicylic acid and 4 grams of acetyl chloride under a reflux condenser, on a water-bath. Continue the heating

as long as HCl gas is evolved, then remove the reflux condenser, and boil off the uncombined acetyl chloride. On cooling the aspirin quickly settles out. Test for free salicylic acid by applying the ferric chloride test. (See Exp. 156.)



160. Ionization of Picric Acid in Aqueous Solutions. In a dry test-tube mix about 0.1 of a gram of picric acid and 5 c.c. of petroleum ether and warm on a water-bath. A colorless solution is obtained due to the fact that picric acid does not ionize in this solvent. Add water and mix thoroughly. Ionization results and a yellow color develops. Draw off the aqueous solution and divide it into two parts. To one part add a little sodium hydroxide solution. Note the deepening of the yellow color. What is the product formed?

161 Properties of Gallic Acid, $C_6H_2—(OH)_3—CO—OH$. *a. Reducing action of gallic acid.* Dissolve 0.5 of a gram of gallic acid in 5 c.c. of hot water and add an equal amount of mixed Fehling's solution. Make note of the result.

b. Action of ferric chloride. Dissolve about 0.5 of a gram of gallic acid in 10 c.c. of water and add 1 or 2 drops of ferric chloride solution. Note the color obtained.

c. Formation of ink from gallic acid. Dissolve 0.5 of a gram of gallic acid in 5 c.c. of hot water and add 3 c.c. of a 10 per cent solution of ferrous sulphate and mix by shaking. With a new pen, write on a piece of paper with some of the solution. To about half of the solution add 2 drops of ferric chloride solution and write with this mixture. Compare the results. Put both papers away until the next day and again compare. Explain what took place.

162. Properties of Tannic Acid. A 1 per cent aqueous solution of tannic acid may be used for these tests.

a. Action of ferric chloride. Add a couple of drops of ferric chloride to 5 c.c. of the tannic acid solution. Explain any change that occurs.

b. Reducing action of tannic acid. Test the reducing action of tannic acid with ammoniacal silver nitrate and with Fehling's solution. Record the results.

c. Precipitating action on alkaloids. To a dilute aqueous solution of quinine hydrochloride add an equal quantity of tannic acid solution.

d. Precipitating action on proteins. Dilute 10 c.c. of skimmed milk with an equal volume of water, and add a few drops at a time, a 1 per cent solution of acetic acid. Stir after each addition of acid. Continue to add the acid in this manner until a flocculent precipitate of casein is obtained. Avoid excess of acid. Filter. Add tannic acid to the clear filtrate. Explain the result.

e. Action of tannic acid on gelatin. Add tannic acid solution to a dilute solution of gelatin. Is the gelatin precipitated?

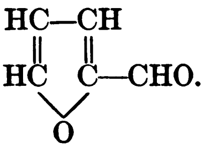
f. Formation of ink from tannic acid. With a little of the tannic acid solution prepare ink according to the technique described for gallic acid. (Exp. 161, *c.*) Do you get a similar result?

CHAPTER XV

HETEROCYCLIC COMPOUNDS

THE term heterocyclic is applied to ring compounds not wholly composed of carbon atoms. One or more links in the closed chain are supplied by other polyvalent elements.

163. Preparation and Properties of Furfuraldehyde. Place 5 grams of bran and 50 c.c. of 2/1 normal hydrochloric acid in a 300- c.c. distilling flask. Connect with a condenser and distil.

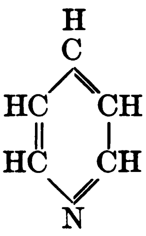


a. Observe the characteristic odor of the distillate. The odor of fresh rye bread is due to the presence of furfuraldehyde.

b. To a few c.c. of the distillate add a drop of aniline and a few drops of concentrated hydrochloric acid. Note the intense red color that develops. Compare Exp. 93.

c. Add a few drops of α-naphthol solution to a little of the distillate and float the mixture on 5 c.c. of concentrated sulphuric acid. Note the play of colors at the junction of the two liquids. Compare Exp. 92.

164. Properties of Pyridine. *a. Reaction of Pyridine.* Add a few drops of pyridine to 5 c.c. of water and test the reaction of the liquid to litmus.



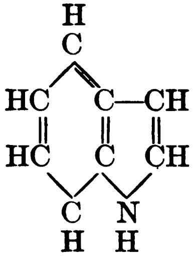
b. Action of ferric chloride. Add a little dilute aqueous solution of pyridine to a solution of ferric chloride. What is the precipitate that forms?

(*c*) *The methyliodide test for pyridine.* Mix 5 drops of pyridine with 5 drops of methyliodide. Heat is evolved, and a solid is formed. (Pyridine methiodide, $C_6H_5N—CH_3I$).

To what class of amines does pyridine belong?

(*d*) *Action of oxidizing agents on pyridine.* Test a small quantity of an aqueous solution of pyridine with Von Baeyer's reagent, concentrated nitric acid, or potassium dichromate and sulphuric acid.

165. Detection of Indole. Indole is one of the products of putrefaction. It may be detected in the products of distillation of putrefied meat as follows:

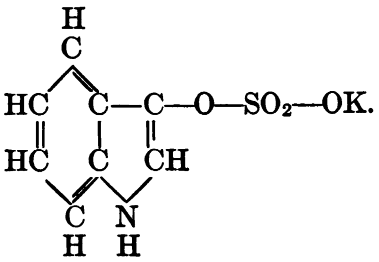


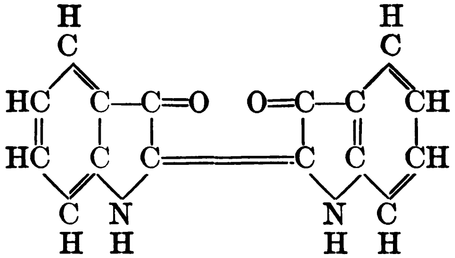
a. Acidify some of the distillate with nitric acid, and add a few drops of sodium nitrite solution. The solution becomes red in color and on standing a red precipitate of nitroso-indole separates out.

b. Formaldehyde reaction (*Konto*). To 1 c.c. of the distillate in a test-tube add 3 drops of formaldehyde solution and 1 c.c. of concentrated sulphuric acid. Shake the mixture and note the appearance of a violet-red color if a trace of indole is present. Repeat with a very dilute aqueous solution of indole (1 : 500,000).

c. Pine wood test for indole. Moisten a pine splinter with concentrated hydrochloric acid and insert it into the solution under examination. The wood becomes cherry-red in color if indole is present.

166. Oxidation of Indican. When indican, which occurs in small quantities in normal urine and in large quantities in certain pathological urines, is treated with acid, it loses its sulphate radical and is converted into indoxyl (hydroxy-indole). If, in addition to the acid, an oxidizing agent is added, the indican will be converted into indigo blue.





a. Place about 10 c.c. of urine in a test-tube. Add an equal volume of HCl, 2 drops of a 1 per cent solution of calcium hypochlorite and 2 to 3 c.c. of carbon tetrachloride or chloroform. Place the thumb over the end of the test-tube and shake the tube thoroughly. The carbon tetrachloride or chloroform will be colored more or less according to the amount of indican present. The color is ordinarily a deep blue, due to the formation of indigo blue; sometimes indigo red is formed instead.

b. Obermeyer's test. To 10 c.c. of urine in a test-tube add 3 to 4 c.c. of carbon tetrachloride and 5 c.c. of Obermeyer's reagent (prepared by adding 2 to 4 grams of ferric chloride [an oxygen carrier] to a liter of concentrated HCl). The result should be the same as in the previous test.

CHAPTER XVI

VEGETABLE ALKALOIDS

The term *vegetable alkaloid* is generally applied to those basic nitrogenous substances which occur in plants, irrespective of any similarity in properties or constitution. They are characterized by their intense action upon the animal body. Some alkaloids, notably coniine and nicotine, are composed of carbon, hydrogen and nitrogen only, and these are soluble, volatile liquids. Most alkaloids, however, are composed of carbon, hydrogen, nitrogen and oxygen. These are crystalline and non-volatile. They are sparingly soluble in water, but they dissolve in most organic solvents. They combine with acids and form salts, which are soluble in water.

The Detection of Alkaloids

(The following scheme, known as the Stas-Otto process for the detection of alkaloids, was condensed from Autenrieth's book, "Detection of Poisons and Powerful Drugs." Authorized translation by W. H. Warren, Ph.D. (Blakiston)).

167. The Isolation of an Alkaloid from a Cadaver, Stomach Content, etc. Put a portion of the finely chopped material into a large flask, and thoroughly mix with two to three times its weight of absolute alcohol. Add enough tartaric acid solution (10 per cent) to give the mixture a distinct acid reaction after it has been shaken. Usually 20 to 30 drops of tartaric acid is sufficient for a laboratory experiment. Avoid excess. Connect the flask, which should be about half full, with a glass tube (80 to 100 cm. long), which serves as a reflux condenser. Heat on a water-bath with frequent shaking, for about 15 minutes. Cool. Filter.

Wash the residue with more alcohol. Evaporate the filtrate, which must have an acid reaction, to a thin syrup in a small beaker on the water-bath. Then mix with 100 c.c. of water. Filter and again evaporate nearly to dryness. Add a little absolute alcohol, and if a precipitate forms, again filter. Again evaporate the filtered, alcoholic solution on a water-bath, and finally dissolve the residue in 50 c.c. of water. Acidify again with tartaric acid and test small portions of the solution with two or more of the "general alkaloidal reagents." (See below.) If precipitates form, proceed according to directions given in experiment 168, et seq.

General alkaloidal reagents. Most alkaloids yield precipitates with the following reagents:

1. Tannic acid;
2. Picric acid;
3. Mercuric chloride;
4. Iodine in potassium iodide;
5. Potassium mercuric iodide;
6. Phosphomolybdic acid;
7. Phosphotungstic acid, etc.

These reagents, termed the general alkaloidal reagents, also give precipitates with amines, purines and other bases, and with proteins. Consequently, they cannot be considered as specific for the alkaloids, but they are useful in detecting the presence of alkaloids or other nitrogenous basic compounds.

NOTE.—If the unknown substance is a powder, dissolve it in distilled water slightly acidified with tartaric acid; then test small portions of the solution with two or more of the alkaloidal reagents; if precipitates form, proceed as indicated in experiment 168 et seq.

A. EXAMINATION OF THE ETHER EXTRACT OF THE TARTARIC ACID SOLUTION

168. Performing the Ether Acid Extraction. Thoroughly extract the acid aqueous solution (see process of prep-

aration described in Exp. 167), two or three times with ether, using each time about the same quantity of the solvent. Use a separating funnel for this purpose. Pour the combined ether extracts into a dry flask loosely stoppered. Retain the aqueous portion for later tests. Slowly evaporate the ether solution in a small glass upon a water-bath which has been previously heated to 38–40° C. Do not have the gas burning during this operation. Add just a little of the ether at a time to avoid the spreading of the alkaloids.

This residue from the acid ether extract might contain the following alkaloids and coal-tar products:

Picrotoxin
Colchicin
Picric acid

Caffeine
Acetanalide
Phenacetine

Antipyrine
Salicylic acid
Veronal

Examine it only for colchicin, caffeine and picrotoxin.

169. Identification of Colchicin. 1. *Sulphuric nitric acid test.* Concentrated H_2SO_4 dissolves colchicin with a yellow color which a drop of HNO_3 will change to green, blue, violet, wine-red and finally back to yellow.

2. *Nitric acid test.* Concentrated nitric acid dissolves colchicin with a dirty violet color which soon changes, when stirred, to brownish-red and finally to yellow. Addition of dilute NaOH solution, until the reaction is alkaline, produces a beautiful orange-yellow or orange-red color. This is the test by which colchicin can be identified.

170. Identification of Caffeine. 1. *Oxidation test.* Pour a few c.c. of saturated chlorine water over caffeine and evaporate the solution to dryness upon the water-bath. A reddish-brown residue will remain. If a few drops of ammonium hydroxide solution are added, a fine purple red color will immediately appear. This test, known as the murexide reaction, is given by several other purines.

2. *Tannic acid test.* Add a little of the tannic acid reagent to an aqueous solution of caffeine. A heavy white

precipitate appears which is soluble in an excess of the acid. This test is not characteristic of caffeine.

171. Identification of Picrotoxin. 1. *H. Melzer's test.* Put a trace of picrotoxin upon a watch-glass and add 1 or 2 drops of a mixture of benzaldehyde and absolute alcohol. Place a drop of concentrated sulphuric acid at the side of the other drops, and tilt the watch-glass so that they will gradually mix. Red streaks will run from the substance through the liquid.

2. *Langley's test.* Mix picrotoxin with three times the quantity of potassium nitrate, and moisten the mixture with 1 *small drop* of concentrated sulphuric acid. Then add strong sodium hydroxide solution in excess and an intense red color will appear.

B. EXAMINATION OF THE ETHER EXTRACT OF THE ALKALINE SOLUTION

(Most of the alkaloids appear here.)

172. Extraction of the Alkaline Solution with Ether. To the aqueous solution obtained in Exp. 167, add enough NaOH solution to make the solution strongly alkaline. The alkali will liberate the alkaloids from their salts and combine with morphine and apomorphine, should they be present. Thoroughly extract this alkaline solution with ether. Place the aqueous solution in a separatory funnel, add an equal volume of ether and shake thoroughly. Repeat this process at least three times. The ether extracts all the alkaloids under these conditions except morphine, apomorphine and narceine. Pour the ether extracts into a dry flask. Retain the aqueous solution.

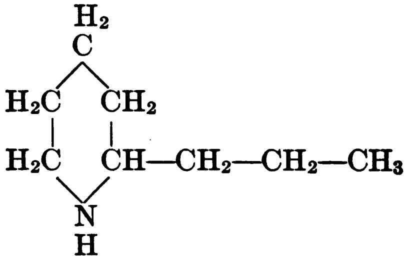
Allow the ether to stand for about an hour; a few drops of water will always separate out. Carefully decant the ether and pour on a small dry dish. The ether should be carefully evaporated, as directed in Exp. 168. The residue, obtained by extracting the alkaline solution with ether, may

contain any alkaloid except morphine, apomorphine or narceine, but especially might it contain the following:

Coniine
Nicotine
Veratrine
Atropine
Codeine
Brucine
Strychnine
Scopolamine
Narcotine
Hydrastine
Pilocarpine
Pyramidone
Quinine
Caffeine
Physostigmine
Cocaine
Antipyrine
Aniline

Examine the solution for the following only: Coniine, atropine, cocaine, codeine, strychnine, brucine and quinine.

173. Identification of Coniine, α-propyl-piperidine.

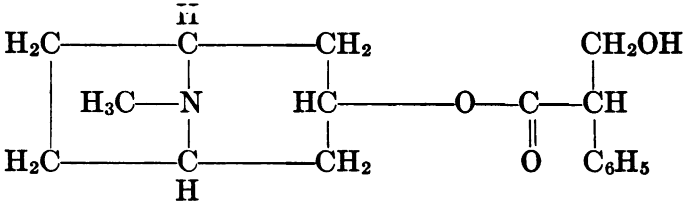


It is a colorless, oily liquid which becomes resinous in contact with air. It possesses an unpleasant, narcotic odor, said to resemble that of mouse urine. Coniine may be recognized by the following tests:

1. *Solubility test.* Dissolve a drop of coniine in just enough cold water to give a clear solution. Gently heat the solution and it will become milky, because coniine is more easily soluble in cold water than it is in hot water. Aqueous coniine solutions have an alkaline reaction.

2. *Crystallization test.* Put a small quantity of coniine upon a watch-glass, or glass slide, and add 1 to 2 drops of HCl. Evaporate to dryness. Coniine hydrochloride will remain. Immediately examine under the microscope. Colorless, or faintly yellow crystals, needle-like or columnar, frequently grouped together in star-shaped clusters, indicate coniine. They show the play of color characteristic of doubly refractive substances.

174. Identification of Atropine, $C_{17}H_{23}NO_3$.



Atropine may be recognized by the following tests:

1. *Vitali's test.* Dissolve the alkaloid in fuming nitric acid, and evaporate to dryness in a porcelain dish upon a water-bath. The residue will be nearly colorless. Moisten the residue when cold with a few drops of a solution of KOH in absolute alcohol. A beautiful violet color will appear and soon a cherry-red. Veratrine behaves in a like manner. Therefore this test is delicate for atropine only in the absence of veratrine.

2. *Odor test.* Heat a small quantity of atropine in a dry test-tube until a white vapor appears. An agreeable odor will be detected at the same time. Then add about 1 c.c. of H_2SO_4, and heat until the acid begins to darken. Dilute at once with about 2 c.c. of water. During the foaming there will be an intense, sweetish odor like that of honey.

175. Identification of Cocaine, $C_{17}K_{21}NO_4$. The structural formula is still a little doubtful. Nearly all the general alkaloidal reagents give precipitates with solutions of cocaine salts. Cocaine may be recognized as follows:

1. *Concentrated acid test.* Concentrated sulphuric and nitric acids dissolve cocaine without color.

2. *Potassium permanganate test.* Add some $KMNO_4$ solution (1 : 100), drop by drop, to a concentrated aqueous solution of a cocaine salt. This reagent will give a violet, crystalline precipitate of cocaine permanganate. In applying the test to the ether residue, dissolve a considerable quantity in 2 drops of dilute HCl and add $KMNO_4$ solution, drop by drop. This is a delicate test for cocaine.

3. *Chromic acid test.* Add a few drops of 5 per cent

chromic acid solution to a solution of cocaine. Each drop will produce a precipitate which will immediately disappear if the solution be shaken. Then add to the clear solution about 1 c.c. of HCl which will produce a more or less crystalline precipitate of orange-yellow cocaine chromate.

176. Identification of Codeine, $C_{17}H_{18}(CH_3)NO_3$. Codeine is the methyl ether of morphine. It crystallizes in colorless, transparent octahedrons, which are often very large.

1. *Sulphuric acid test.* Strong H_2SO_4 dissolves codeine without color. After long contact or upon application of gentle heat, the solution will have a reddish to violet-red color.

2. *Test with Froehde's reagent.* (This is a solution of molybdic acid in 1 c.c. of hot, concentrated, pure H_2SO_4. This solution should be colorless. It does not keep long.) Froehde's reagent dissolves codeine with a yellowish color, which soon changes to green and later to blue. Gentle warming will hasten this change of color.

177. Identification of Strychnine, $C_{21}H_{22}O_2$. Strychnine crystallizes in colorless, shining prisms belonging to the rhombic system.

The sulphur acid dichromate test for strychnine. Dissolve a very small quantity of strychnine in 2 or 3 drops of strong H_2SO_4 upon a watch-glass. The solution should be colorless. Add a fragment of $K_2Cr_2O_7$ and hold it firmly in one place upon the glass; intense blue or blue-violet streaks will come from the potassium dichromate, if the watch-glass be moved up and down. If the entire mixture is stirred the sulphuric acid will be colored a beautiful blue or blue-violet.

178. Identification of Brucine, $C_{23}H_{26}N_2O_4$. Brucine crystallizes from dilute alcohol in colorless, transparent plates

1. *Nitric acid-stannous chloride test.* Strong nitric acid dissolves brucine and its salts with a blood-red color This color, however, is not stable and soon changes to yellowish-red and finally, especially with heat. to yellow. Add a few drops of freshly prepared dilute stannous-chloride solution

to this yellowish-red or yellow solution. An intense violet color will appear.

2. *Nitric acid ammonia test.* Add a few drops of strong HNO_3 to brucine upon a watch-glass and evaporate the solution to dryness. An orange-red to brownish residue will remain. Ammonia will change this color to grass-green. Much strychnine obscures the delicacy of this test.

179. Detection of Strychnine and Brucine when Together. Strong H_2SO_4 dissolves a mixture of these two alkaloids without color. Addition of a trace of nitric acid or a fragment of KNO_3 will produce the red characteristic of brucine. This color will soon change to yellow. Then add a small crystal of $K_2Cr_2O_7$ and stir. The mixture will become blue or reddish-violet if strychnine is present.

180. Identification of Quinine. $C_{20}H_{24}N_2O_2$. 1. *Fluorescence test.* Dissolve the residue from the ether extraction of the alkaline solution in a small quantity of dilute H_2SO_4. This solution will show a blue fluorescence when quinine is present.

2. *Thalleioquine test.* Dissolve quinine in a few drops of very dilute acetic acid, and add 5 to 10 drops of saturated chlorine water. The solution will either be colorless or have a faint blue fluorescence. Addition of NH_4OH in excess will produce an emerald green color.

C. EXTRACTION OF THE AMMONIACAL SOLUTION WITH ETHER AND CHLOROFORM

181. Carefully neutralize the alkaline aqueous solution obtained in Exp. 172 with hydrochloric acid and then make it strongly alkaline with ammonium hydroxide. This ammoniacal solution should then be treated as follows:

a. Extract with ether. The solution should be shaken up with ether in a separatory funnel. On evaporating the residue from this ether extract one will obtain any apomorphine that may be present. Traces of morphine will also appear in this residue.

b. Extract with chloroform. This extract will contain morphine and narceine should they be present.

182. Identification of Apomorphine, $C_{17}H_{17}NO_2$. Apomorphine is freely soluble in alcohol, ether, amyl alcohol and chloroform.

Apomorphine may be recognized by the following tests:

1. *Husemann's test.* Dissolve apomorphine in a strong H_2SO_4 solution and add a drop of strong HNO_3. An evanescent violet will appear and soon change to blood-red and later to yellowish-red.

2. *Pellagri's test.* Dissolve apomorphine in dilute HCl or H_2SO_4 and then neutralize with $NaHCO_3$. Then add 1 to 4 drops of an alcoholic solution of iodine and shake thoroughly for several minutes. The solution will have a blue-green color. Extract with ether and this solvent will have a violet color.

3. *Test with Froehde's reagent* (*see Exp.* 176, 2). This reagent dissolves pure apomorphine with a green color. When the alkaloid has been acted upon by air to any extent, the color will be violet.

183. Identification of Morphine, $C_{17}H_{19}NO_3$. 1. *Sulphuric acid-iodic acid chloroform test.* To a portion of ammoniacal solution add a little dilute H_2SO_4, then a few c.c. of iodic acid solution and finally a small quantity of chloroform. Shake the mixture vigorously. A violet color in the chloroform caused by free iodine indicates morphine.

2. *Nitric acid test.* Strong HNO_3 dissolves morphine with a blood-red color which gradually changes to yellow. Stannous chloride solution does not produce a violet color in this solution. (Distinction from brucine.)

3. *Ferric chloride test.* Add 1 to 2 drops of *neutral* ferric chloride solution to a neutral solution of a morphine salt. A beautiful blue color will appear.

4. *Silver test.* Warm a solution of a morphine salt with $AgNO_3$ solution and an excess of NH_4CH. Morphine will produce a gray precipitate of metallic silver.

CHAPTER XVII

PROTEINS

184. Detection of the Elements Contained in Proteins. *a. Detection of carbon and hydrogen.* Take a small quantity of dry protein (casein or egg-albumin) and test for carbon and hydrogen according to the technique outlined in Exp. 1.

b. Detection of nitrogen and sulphur. Heat a few c.c. of egg-albumin solution with an equal volume of strong sodium hydroxide solution. Hold a piece of moist, red litmus paper in the fumes. Explain.

Dilute this solution with about two volumes of water, and add several drops of lead acetate solution. Explain.

c. Detection of phosphorus. Place about ¼ gram of casein and 5 c.c. of concentrated nitric acid in an evaporating dish and boil until nearly dry. Add 10 to 15 c.c. of water and a little ammonium molybdate solution.

d. Detection of iron. Mix a small quantity of dried blood in a crucible with three to four times its quantity of fusion mixture and heat until all the organic matter has been oxidized. Cool. Dissolve the residue in dilute HCl; filter; and test the filtrate for ferric iron by adding potassium ferrocyanide.

Does iron occur in many proteins?

NOTE.—Nitrogen, sulphur and phosphorus may also be detected after fusion with sodium. See Exp. 2, 4 and 5.

185. Preparation of Egg-albumin Solution. Carefully segregate the white of an egg from the yolk. Place the white in an evaporating dish and cut it with a pair of scissors; add a little cold water and continue the cutting with the scissors. Continue to add water until about ten times the volume of the egg-white has been added. Filter first through muslin and then through filter paper.

A nearly colorless solution containing about 1 per cent of protein is obtained by this process.

PRECIPITATION REACTIONS OF PROTEINS

186. Influence of Strong Mineral Acids. *a.* Put about 5 c.c. of strong nitric acid in a small test-tube. Hold the tube at an angle of 45° and by means of a pipette allow a few c.c. of egg albumin solution to flow slowly down the side of the tube. Note the precipitate which is formed at the junction of the two liquids. This is known as Heller's ring test for proteins.

Repeat, using strong hydrochloric acid, also sulphuric acid.

Record your results.

187. Influence of Strong Organic Acids. Add a few drops of glacial acetic acid to a little egg albumin solution. Is the protein precipitated?

188. Precipitation by Salts of the Heavy Metals. *a. Mercuric chloride.* Add 1 to 2 drops of mercuric chloride solution to a little egg albumin solution. Note what happens. Add some saturated sodium chloride solution. Does any change take place? Now add a few drops of dilute hydrochloric acid. Again note what happens.

Record your results.

b. Copper sulphate. Add copper sulphate solution, drop by drop, to a little egg albumin solution. Note the color of the solution formed. Add some sodium hydroxide solution. Does the color change?

To a little egg albumin solution add some Fehling's solution. A violet color is produced. How, then, could one detect a reducing sugar in a solution also containing albumin?

c. Lead acetate. Add a few drops of lead acetate to some egg albumin solution. Note the nature of the precipitate. Egg albumin solution or milk is used as an antidote for lead or mercury poisoning. Why?

d. Ferric chloride. To a little egg albumin solution in a test-tube add ferric chloride solution, drop by drop, until

an excess of the reagent has been added, noting any changes which may occur.

189. Precipitation by Alkaloidal Reagents in Acid Solutions. *a. Potassium mercuric iodide.* Add to 3 or 4 c.c. of egg albumin solution 1 drop of dilute hydrochloric acid and 2 drops of the potassium mercuric iodide solution.

b. Phosphotungstic acid. Slightly acidify a solution of egg albumin with either hydrochloric acid or sulphuric acid and then add a little phosphotungstic acid reagent.

c. Phospho-molybdic acid. Follow the same technique outlined in *b*, using phospho-molybdic acid instead.

d. Hydroferrocyanic acid. To 5 c.c. of egg albumin solution in a test-tube add a few drops of acetic acid. Shake; and then add, drop by drop, potassium ferrocyanide solution.

This test is often used clinically for detecting protein in urine. The test is not so delicate in the presence of netural salts.

e. Trichloracetic acid. To a little egg albumin solution in a test-tube add an equal volume of a 10 per cent solution of trichloracetic acid.

f. Picric acid. Add picric acid, drop by drop, to a little egg albumin solution, which has previously been made acid with very dilute acetic acid.

Record your results in each of the above tests.

190. Action of Alcohol. *a.* Put 3 c.c. of egg albumin solution in a test-tube and add 10 c.c. of alcohol. Remove a small portion of the precipitate and test its solubility in water. Allow the remainder of the precipitate to remain in contact with the alcohol until the next laboratory session. At that time again test its solubility in water. Do you obtain the same results? Explain.

b. To 5 c.c. of alcohol add 1 drop of 0.2 per cent HCl, then add 1 c.c. of egg albumin solution.

c. To 5 c.c. of alcohol add 1 drop of dilute NaOH, then add 1 c.c. of egg albumin solution.

If any precipitate forms in *b* or *c*, test its solubility in water as in *a*. Compare your results.

191. Coagulation by Heat. *a.* Heat 5 c.c. of egg albumin solution in a test-tube to boiling. Note any change in the appearance of the solution. Is there a definite coagulum?

b. To 5 c.c. of egg albumin solution in a test-tube add 2 drops of dilute acetic acid. Then heat to boiling. Do you get a coagulum?

c. Repeat *b*, using 2 drops of glacial acetic acid instead of the dilute. Do you get the same results as in the previous test?

d. Heat 5 c.c. of egg albumin solution after adding 3 drops of 10 per cent $NaCO_3$. Do you get a coagulum?

192. Coagulation Temperature. Add 2 drops of dilute acetic acid to 5 c.c. of egg albumin solution in a test-tube. Put a thermometer in the solution and place the test-tube in a beaker of water. Heat the water and determine carefully the temperature at which coagulation occurs. Both the water in the beaker and the solution in the tube should be gently stirred from time to time. The solution becomes cloudy just before it coagulates.

193. Color Reactions of Proteins. *a. Biuret reaction.* Mix 2 to 3 c.c. of the egg albumin solution with 5 c.c. of 8 per cent sodium hydroxide solution and 2 drops of a 0.5 per cent solution of copper sulphate. *Caution.* Always use the same quantity of the alkali and of the copper sulphate, as variations in the amounts of the copper sulphate will lead to errors in your conclusions.

Gies' biuret reagent. Gies has recently devised a reagent which overcomes the above-mentioned difficulties. This reagent consists of 8 per cent NaOH solution, to which enough dilute copper sulphate solution has been added to impart a slight blue color to the liquid. The copper sulphate must be added a drop or two at a time, with thorough shaking after each addition. When using this reagent proceed as follows: Take two clean test-tubes and put 2 to 3 c.c. of the reagent in each. To one test-tube add an equal volume of the egg albumin solution and to the other add

an equal volume of water. The tube containing the protein should have a distinct violet or pink color, whereas the solution in the other (control) tube is pale blue.

This reaction is due to the presence of at least two —CO—NH— groups. (See Exp. 78, *d*, and 188, *b*.)

b. Millon's reaction. To a little of the egg albumin solution add a few drops of Millon's reagent (solution of mercurous and mercuric nitrates). A precipitate forms, which, on heating, becomes brick-red. The red color constitutes the essential part of the test.

On what group in the protein molecule does this test depend? See Exp. 145, *c*, and 156.

Repeat this test after adding some sodium chloride to the egg albumin. What takes place? Explain.

c. Xanthoproteic reaction. To 4 to 5 c.c. of the egg albumin solution add 2 c.c. of concentrated nitric acid. A white precipitate is produced (this white precipitate is not produced with all proteins or if the protein solution is too weak). Boil. The precipitate or liquid turns yellow. Cool, and render distinctly alkaline with ammonium hydroxide. An orange color is produced.

On what group in the protein molecule does this test depend? See Exp. 145, *d*.

d. Hopkins-Cole reaction. Put 2 to 3 c.c. of egg albumin solution and an equal volume of Hopkins-Cole reagent (an aqueous solution of glyoxylic acid), in a test-tube and mix thoroughly. In a second tube put 5 c.c. of concentrated sulphuric acid. Hold this tube at an angle of 45° and by means of a pipette allow the albumin-glyoxylic acid mixture to run slowly down the side. A reddish-violet color forms at the junction of the two liquids. This color is due to the presence of the *tryptophane group* in the protein molecule.

e. Apply the Molisch test (Exp. 92), to a few c.c. of concentrated egg-white solution. If no color is produced, set the tube aside for a few minutes and again examine. If a positive test is obtained, what group must be present in the molecule?

194. Separation of Proteins by Means of Neutral Salts. For these experiments 1 part egg white is dissolved in 10 parts of 1 per cent NaCl.

a. Separation of a globulin from an albumin by means of sodium chloride. Add to 10 c.c. of egg-white solution in a small beaker an equal volume of a saturated solution of sodium chloride. Do you obtain a precipitate? Add finely powdered sodium chloride to this mixture until the solution is saturated and a slight residue of sodium chloride remains (this will require 3.6 grams of the solid). The globulin is precipitated. Filter the solution; add to the filtrate 1 drop of 2 per cent acetic acid and heat to boiling. A coagulum indicates albumin.

b. Separation of a globulin from an albumin by means of magnesium sulphate. Follow the same technique outlined in *a*, using $MgSO_4$ instead of NaCl. Do you get the same results?

c. Separation of a globulin from an albumin by means of ammonium sulphate. Add to 10 c.c. of egg-white solution an equal volume of a saturated ammonium sulphate solution. The globulin is precipitated. Filter, and divide the filtrate into two parts. To part one add 1 drop of a 2 per cent acetic acid solution and boil. A coagulum indicates albumin. To part two of the filtrate, add solid ammonium sulphate to point of saturation. (Since this solution is half saturated, about 4 grams will be required to effect saturation of 10 c.c.) The albumin is precipitated. Filter, and test the filtrate for protein with biuret reagent.

195. Preparation and Properties of Alkali Metaprotein. Add to 10 c.c. of egg-white solution 1 c.c. of 10 per cent sodium hydroxide solution. Place the test-tube in a water-bath at 50° C. and allow to stand for 30 minutes; the protein is partly converted into alkali-metaprotein. Heat a small portion of the solution to boiling. Does it coagulate? Neutralize another portion with 0.2 per cent HCl. The metaprotein is precipitated. Avoid excess of acid. Put a portion of the precipitate in distilled water. Is it

soluble? Test the solubility of another portion in 2 per cent acetic acid.

196. Preparation and Properties of Acid-metaprotein. Dissolve 1 gram of dried egg-white in 10 c.c. of 0.4 per cent HCl and keep in an incubator at 38° C. for 24 hours. If the solution is not perfectly clear, filter. Heat a small portion of the solution to boiling. Does it coagulate? Carefully neutralize the remainder of the solution with 0.5 per cent $NaCO_3$ solution. Then test the precipitated acid-metaprotein as follows: *a.* Solubility in distilled water; *b.* Millon's reaction· *c* Hopkins-Cole reaction; *d.* Biuret reaction.

197. Separation of the Products of Acid Hydrolysis of Proteins. This separation scheme may be applied to a portion of the above acid metaprotein solution or to a filtered solution of Witte's peptone (5 per cent).

Carefully neutralize. If a precipitate appears, acid metaprotein is present. Filter, and heat the filtrate to boiling. If a precipitate appears, unaltered coagulable protein is present.

Filter, if necessary, and half saturate with ammonium sulphate. (Add an equal volume of saturated ammonium sulphate solution.) If a precipitate appears, primary proteose is present.

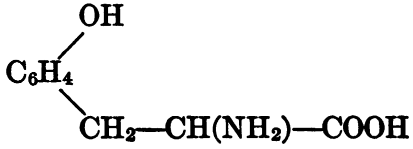
Filter; saturate the filtrate with solid ammonium sulphate and heat to boiling.

If a precipitate appears at this point secondary proteoses are present.

Filter, if necessary, and apply the biuret and xanthoproteic tests to the filtrate.

If the tests are positive, peptones are present.

198. Isolation of a Typical Amino-acid from a Protein.



Tyrosin $C_6H_4\begin{matrix}/OH\\ \backslash CH_2—CH(NH_2)—COOH\end{matrix}$ *from casein.*

Dissolve 100 grams of casein in 1 liter of 0.1 normal Na_2CO_3, add 1 gram of trypsin, 5 c.c. of toluene (to prevent putrefaction), shake well, and keep in a constant temperature bath at 37° C. for 7 or 8 days. The solution gradually becomes cloudy and tyrosin settles out. Filter. Dissolve the precipitate in 0.1 normal HCl, decolorize by boiling with finely pulverized animal charcoal and filter. Cool and carefully neutralize with ammonia. Crystals of fairly pure tyrosin may be obtained by this method.

199. Properties of Tyrosin. Make the following tests on the tyrosin crystals obtained in the last experiment:

a. Microscopical examination. Place a minute crystal of tyrosin on a slide, add a drop of 0.1 normal NH_4OH, and as soon as the tyrosin has dissolved allow the ammonia to evaporate by holding the slide above a Bunsen flame. The tyrosin crystallizes in characteristic bunches of fine needles. Make a drawing of those you obtain.

b. Solubility. Test the solubility of very small amounts of tyrosin in cold and hot water, cold and hot 95 per cent alcohol, dilute NH_4OH, dilute NaOH and dilute HCl.

c. Color tests. Dissolve a small amount of the tyrosin crystals in a few c.c. of water and then perform the protein color tests, i.e., repeat Exp. 193 with this solution. Make careful notes of which ones are positive.

d. Mörner's test. Add about 2 c.c. of Mörner's reagent (1 volume formalin, 45 volumes water, and 55 volumes concentrated H_2SO_4), to a little of the tyrosin in a test-tube, and *gradually* raise the temperature to the boiling-point. A green color results.

e. Reaction with copper carbonate. Add a little copper carbonate to a few c.c. of the tyrosin solution and boil. Do you get the blue color obtained with aliphatic amines? (See Exp. 74, *d.*)

200. Preparation of a Crystalline Protein. *Edestin from hemp-seed.* Carefully grind about 25 grams of hemp-seed in a mortar. Put only a small amount of the seed in the mortar at a time and add a few grains of sand with each

addition of the seed. Transfer the crushed seed to a casserole containing about 200 c.c. of 5 per cent NaCl solution, which has been heated to *exactly 60° C.* Keep the temperature at 58-60° for one-half hour. This can be accomplished by keeping a very low flame under the casserole and stirring occasionally with the thermometer. If the temperature rises above 60°, remove the flame. Filter hot into a *warm beaker* and through a *warm funnel* containing a fluted filter paper. The beaker and funnel should be warmed as follows: In another beaker heat about 100 c.c. of 5 per cent NaCl to about 70°; pour this through the funnel into the beaker that will be used to collect the edestin solution; then pour it back into the first beaker. The supernatant liquid of the saline extract of hemp-seed should now be transferred to the warm funnel and the funnel should be covered with a watch-glass to prevent loss in heat from the solution. Finally transfer all the solid mass to the funnel and wash it with the saline solution that was used to warm the beaker and funnel. At the time of washing, this solution should be just 60°. Set the filtrate aside to cool slowly.

At the next laboratory period, decant off most of the liquid carefully and filter off the solid. Retain the precipitate and the filtrate.

201. Properties of Edestin. *a. Microscopical examination.* Place a very small amount of the precipitate obtained in the last experiment on a glass slide; cover; and examine under a microscope. Make a sketch of the crystals.

b. Solubility. Test the solubility of edestin in the "biological solvents" (see Exp. 91). Record results.

c. Protein color tests. Apply the following tests to the edestin crystals: Xanthoproteic, Millon's biuret, and Hopkins-Cole. Also test for sulphur. Record your results.

d. Coagulation test. Try the coagulability of edestin in a neutral solution by placing a small amount of the crystals in a test-tube and heating to boiling. Now add a few drops of dilute HCl. Is there any change?

Apply the following tests to the filtrate obtained in Exp. 200.

e. Influence of protein precipitants. Test with nitric acid, trichloracetic acid, tannic acid, picric acid, lead acetate, etc.

f. Saturation with sodium chloride. Saturate some of the filtrate with solid NaCl. What happens? How does this result compare with that obtained upon saturating albumin solutions?

g. Effect of dilution. Place some of the filtrate in a large beaker and add 10 or 15 volumes of water. What happens? From the foregoing tests, what kind of a protein would you judge edestin to be?

202. Preparation of a Prolamine. (Gliadin from wheat.) To 50 grams of wheat flour gradually add enough water to make a stiff dough (about 25 c.c.). Knead the dough with your hands for 2 minutes, then let it stand for half an hour. At this time knead the dough in a piece of cheesecloth under a stream of cold water until all the starch is washed out. The sticky mass that is left is a mixture of gliadin and glutenin known as *gluten*. Cut the gluten into small pieces with a knife or scissors. Transfer the pieces to a small flask containing 100 c.c. of 70 per cent alcohol. Extract the gliadin by boiling the mixture on the steam-bath for 30 minutes. Filter hot. Repeat the extraction process with another 100 c.c. of 70 per cent alcohol. Again filter hot. Combine the filtrates and evaporate to about one-fourth of the original volume. Cool and then add 10 c.c. of 2/1 normal NaCl solution little by little with constant stirring. Allow the precipitate to settle out, filter off and dehydrate with cold 95 per cent alcohol. Spread out on a watch-glass and dry in a desiccator.

203. Properties of Gliadin. Make the following tests on the gliadin obtained in the last experiment.

a. Solubility. Test the solubility of very small amounts of gliadin in 5 c.c. portions of the "biological solvents" (Exp. 91), and in hot and cold absolute alcohol. Record your results.

b. Protein color tests. Dissolve the remainder of your gliadin preparation in 0.1 normal HCl and then perform the protein color tests (Exp. 178). Record your observations.

204. Properties of Gelatin. Gelatin, although often classed with the albuminoids (a class of proteins that are characterized being soluble in all neutral solvents), is in reality a derived protein. It is formed by partial hydrolysis of a typical albuminoid, *collagen,* which is found in tendon, cartilage and bone.

a. Solubility of gelatin. Cut a piece of sheet-gelatin into small pieces (1 cm. square). Place one piece in 5 c.c. portions of each of the "biological solvents" (Exp. 91), and let the tubes stand for 5 minutes. Note any change in the appearance of the gelatin in the tubes. In another tube place two pieces of gelatin and 5 c.c. of water; heat gently by placing the tube in hot water. In the hot water the gelatin dissolves. Cool. It will gel. This is the best test for gelatin. (Solutions of less than 1 per cent concentration do not gel readily.)

For the following tests a 1 per cent solution of gelatin is supplied.

b. Protein color tests. Repeat Exp. 193 with portions of the gelatin solution. Some of the color tests are positive with gelatin, others are not. Make a record of your results.

c. Test for sulphur. Repeat Exp. 184, *b.* Is sulphur present? From the results you obtained in this test and in the color tests, what do you conclude in regard to the composition of gelatin?

d. Precipitation test. Is gelatin precipitated by half saturating the solution with ammonium sulphate (use 5 c.c. of the gelatin solution and 5 c.c. of saturated ammonium sulphate)? By 95 per cent alcohol? By lead acetate? By picric acid? By tannic acid?

e. Effect of boiling in acid solution. Dissolve 1 gram of gelatin in 10 c.c. of warm water; add 1 c.c. of 2/1 normal HCl and boil for 5 minutes. Cool the tube under the tap.

If it does not gel, neutralize with 2/1 normal NaOH solution, and observe if it gels. If the gelatin should gel at this point, reacidify and heat again for 2 minutes; cool; neutralize and test its ability to gel. Would excessively acid fruit and too long boiling interfere with the making of jellies?

205. Solubility of the Albuminoids (Scleroproteins). Test the solubility of hair, finger-nails and horn shavings in the "biological solvents" (Exp. 91). Record your results.

APPENDIXES

APPENDIX A—TABLES

TABLE I. INTERNATIONAL ATOMIC WEIGHTS, 1916

	Symbol	Atomic weight		Symbol	Atomic weight
Aluminum	Al	27.1	Molybdenum	Mo	96.0
Antimony	Sb	120.2	Neodymium	Nd	144.3
Argon	A	39.88	Neon	Ne	20.2
Arsenic	As	74.96	Nickel	Ni	58.68
Barium	Ba	137.37	Nitrogen	N	14.01
Bismuth	Bi	208.0	Osmium	Os	190.9
Boron	B	11.0	Oxygen	O	16.00
Bromine	Br	79.72	Palladium	Pd	106.7
Cadmium	Cd	112.40	Phosphorus	P	31.04
Caesium	Cs	132.81	Platinum	Pt	195.2
Calcium	Ca	40.07	Potassium	K	39.10
Carbon	C	12.00	Praseodymium	Pr	140.9
Cerium	Ce	140.25	Radium	Ra	226.0
Chlorine	Cl	35.46	Rhodium	Rh	102.9
Chromium	Cr	52.0	Rubidium	Rb	85.45
Cobalt	Co	58.97	Ruthenium	Ru	101.7
Columbium	Cb	93.5	Samarium	Sa	150.4
Copper	Cu	63.57	Scandium	Sc	44.1
Dysprosium	Dy	162.5	Selenium	Se	79.2
Erbium	Er	167.7	Silicon	Si	28.3
Europium	Eu	152.0	Silver	Ag	107.88
Fluorine	F	19.0	Sodium	Na	23.00
Gadolinium	Gd	157.3	Strontium	Sr	87.63
Gallium	Ga	69.9	Sulfur	S	32.06
Germanium	Ge	72.5	Tantalum	Ta	181.5
Glucinum [1]	Gl	9.1	Tellurium	Te	127.5
Gold	Au	197.2	Terbium	Tb	159.2
Helium	He	4.00	Thallium	Tl	204.0
Hydrogen	H	1.008	Thorium	Th	232.4
Indium	In	114.8	Thulium	Tm	168.5
Iodine	I	126.92	Tin	Sn	118.7
Iridium	Ir	193.1	Titanium	Ti	48.1
Iron	Fe	55.84	Tungsten	W	184.0
Krypton	Kr	82.92	Uranium	U	238.2
Lanthanum	La	139.0	Vanadium	V	51.0
Lead	Pb	207.20	Xenon	Xe	130.2
Lithium	Li	6.94			
Lutecium	Lu	175.0	Ytterbium (Neoytterbium)	Yb	173.5
Magnesium	Mg	24.32	Yttrium	Yt	88.7
Manganese	Mn	54.93	Zinc	Zn	65.37
Mercury	Hg	200.6	Zirconium	Zr	90.6

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[1] Also called Beryllium, Be.

TABLE II. SPECIFIC GRAVITY AND PERCENTAGE OF ALCOHOL

(According to Squibb.)

Per cent alcohol by volume	Sp. Gr. at $\frac{25^\circ}{15.56}$ C.	Per cent alcohol by volume	Sp. Gr. at $\frac{25^\circ}{15.56}$ C.
1	0.9970	51	0.9246
2	.9953	52	.9226
3	.9938	53	.9205
4	.9922	54	.9184
5	.9909	55	.9164
6	.9893	56	.9143
7	.9876	57	.9122
8	.9868	58	.9100
9	.9855	59	.9081
10	.9846	60	.9056
11	.9831	61	.9034
12	.9816	62	.9011
13	.9801	63	.8989
14	.9793	64	.8969
15	.9787	65	.8947
16	.9773	66	.8923
17	.9759	67	.8895
18	.9746	68	.8870
19	.9733	69	.8846
20	.9726	70	.8821
21	.9719	71	.8796
22	.9706	72	.8771
23	.9692	73	.8746
24	.9678	74	.8719
25	.9668	75	.8689
26	.9655	76	.8665
27	.9646	77	.8641
28	.9631	78	.8616
29	.9617	79	.8583
30	.9603	80	.8558
31	.9594	81	.8530
32	.9582	82	.8500
33	.9567	83	.8476
34	.9556	84	.8444
35	.9538	85	.8414
36	.9521	86	.8384
37	.9507	87	.8352
38	.9489	88	.8326
39	.9473	89	.8291
40	.9456	90	.8258
41	.9438	91	.8223
42	.9424	92	.8191
43	.9402	93	.8156
44	.9382	94	.8118
45	.9363	95	.8083
46	.9343	96	.8044
47	.9323	97	.8003
48	.9307	98	.7960
49	.9288	99	.7914
50	.9267	100	.7865

TABLE III. TABLE OF FREEZING MIXTURES OF POWDERED ICE AND VARIOUS SALTS

100 parts of powdered ice at —1° mixed with the indicated weight of the following substances will give the temperature shown in the third column.

Substance	Parts by weight	Temperature deg.
Potassium sulphate	10	−1.9
Sodium carbonate crystals	20	−2.0
Potassium nitrate	13	−2.85
Potassium chloride	30	−10.9
Ammonium chloride	25	−15.4
Ammonium nitrate	45	−16.75
Sodium nitrate	50	−21.3
Sodium chloride	30	−22.4
Calcium chloride crystals ($CaCl_2+2H_2O$)	143	−50.

APPENDIX B. REAGENTS AND SOLUTIONS.

ACIDS

	100 c.c. contains
Acetic acid, glacial, sp. gr. 1.06	111.1 gms. CH_3COOH
Acetic acid, dilute, 1/2 normal (27 c.c. glacial acetic acid made up to 1 liter.)	3.0 gms. CH_3COOH
Hydrochloric acid, conc., sp. gr. 1.19	36.6 gms. HCl
Hydrochloric acid, dilute, 2/1 normal (200 c.c. conc. acid made up to 1 liter.)	7.3 gms. HCl
Hydrochloric acid, dilute, 1/10 normal (10 c.c. of conc. HCl made up to 1 liter.)	0.36 gm. HCl
Nitric acid, conc., sp. gr., 1.42	99.1 gms. HNO_3
Nitric acid, dilute, 2/1 normal (125 c.c. conc. acid made up to 1 liter.)	12.6 gms. HNO_3
Sulphuric acid, conc., sp. gr. 1.84	175.9 gms. H_2SO_4
Sulphuric acid, dilute, 2/1 normal (56 c.c conc. acid made up to 1 liter.)	9.8 gms. H_2SO_4
Sulphuric acid, dilute, 1/10 normal (2.8 c.c. conc. acid made up to 1 liter.)	0.5 gm. H_2SO_4

ALKALIES

	100 c.c. contains
Ammonia, conc., sp. gr. 0.88	31.0 gms. NH_3
Ammonia, dilute, 2/1 normal (105 c.c. conc. ammonia made up to 1 liter.)	3.4 gms. HN_3
Barium hydroxide, 1/4 normal (40 gms. $Ba(OH)_2-8H_2O$ dissolved in water and made up to 1 liter.)	4.2 gms. $Ba(OH)_2$
Sodium hydroxide, sp. gr. 1.34 (410 gms. 98% caustic soda dissolved in water and made up to 1 liter.)	40.0 gms. NaOH
Sodium hydroxide, dilute, 2/1 normal (82 gms. 98% caustic soda dissolved in water and made up to 1 liter.)	8.0 gms. NaOH
Sodium hydroxide, dilute, 1/10 normal (4.1 gms. 98% caustic soda dissolved in water and made up to 1 liter.)	0.4 gm. NaOH

SALT SOLUTIONS

Ammonium chloride, 2/1 normal (107 gms. NH_4Cl dissolved in water and made up to 1 liter.)	10.7 gms.
Ammonium carbonate, 1/1 normal (48 gms. $(HN_4)_2CO_3$ dissolved in water and made to 1 liter.)	4.8 gms.

	100 c.c. contains
Ammonium molybdate (124 gms. $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ dissolved in cold water and made up to 1 liter.)	11.5 gms.
Barium chloride, 1/1 normal (122 gms. $BaCl_2\cdot 2H_2O$ dissolved in water and made up to 1 liter.)	10.4 gms.
Calcium chloride, 1/1 normal (110 gms. $CaCl_2\cdot 6H_2O$ dissolved in water and made up to 1 liter.)	5.5 gms.
Copper sulphate (Fehling's solution A, about one-half normal (69.28 gms. $CuSO_4\cdot 5H_2O$ dissolved in water and made up to 1 liter.)	4.0 gms.
Ferric chloride, 1/2 normal (27 gms. dissolved in water and made up to 1 liter.)	2.7 gms.
Iodine solution, 1/10 normal (13 gms. of iodine are dissolved in a solution of 30 gms. KI in 250 c.c. of water and made up to 1 liter.)	1.3 gms.
Lead acetate, 1/2 normal (95 gms. $Pb(C_2H_3O_2)_2\cdot 3H_2O$ dissolved in water and made up to 1 liter.)	8.1 gms.
Lead acetate, basic, 1/2 normal (56 gms. litharge dissolved in a solution of 95 gms. lead acetate in about 800 c.c. water and made up to 1 liter.)	13.6 gms.
Potassium bichromate, 1/2 normal (24.55 gms. $K_2Cr_2O_7$ dissolved in water and made up to 1 liter.)	2.4 gms.
Potassium forrocyanide, 1/2 normal (5.3 gms. $K_4Fe(CN)_6\cdot 3H_2O$ dissolved in water and made up to 1 liter.)	4.6 gms.
Potassium ferricyanide, 2/1 normal (44 gms. $K_3Fe(CN)_6$ dissolved in water and made up to 1 liter.)	4.4 gms.
Potassium permanganate, 1/20 normal (1.58 gms. $KMnO_4$ dissolved in water and made up to 1 liter.)	0.16 gm.
Mercuric chloride, 1/2 normal (68 gms. $HgCl_2$ dissolved in water and made up to 1 liter.)	6.8 gms.
Sodium carbonate, 1/10 normal (14.3 gms. $NaCO_3\cdot 10H_2O$ dissolved in water and made up to 1 liter.)	0.5 gm.
Sodium chloride, 2/1 normal (117 gms. NaCl dissolved in water and made up to 1 liter.)	11.7 gms.
Silver nitrate, 1/10 normal (17 gms. $AgNO_3$ dissolved in water and made up to 1 liter.)	1.7 gms.
Sodium nitrite, 1/10 normal (6.9 gms. $NaNO_2$ dissolved in water and made up to 1 liter.)	0.7 gm.

	100 c.c. contains
Sodium phosphate, 1/1 normal .... (119.5 gms. $Na_2HPO_4 \cdot 12H_2O$ dissolved in water and made up to 1 liter.)	4.7 gms.
Uranium acetate, 1/10 normal .... (21.3 gms. $UrO_2(C_2H_3O_2)_2 \cdot 2H_2O$ dissolved in water and made up to 1 liter). (1 c.c.= 0.00355 gm. P_2O_5).	2.1 gms.

SPECIAL REAGENTS

Acid sodium acetate solution.
100 gms. sodium acetate
30 c.c. glacial acetic acid
} are dissolved in water and made up to 1 liter.

Alcoholic caustic soda.
20 gms. sodium or 20 gms. caustic soda.
} are dissolved in alcohol and made up to 1 liter.

Ammonium sulphate. (Saturated solution.)
780 gms. ammonium sulphate are dissolved in water and made up to 1 liter.

Barfoed's reagent.
66 gms. copper acetate, 10 c.c. glacial acetic acid are dissolved in water and made up to 1 liter.

Benedict's qualitative reagent for glucose, etc.
173 gms. sodium citrate
100 gms. anhydrous sodium carbonate
} are dissolved in about 600 c.c. water and filtered into a 1-liter measuring cylinder and diluted to about 850 c.c.

17.3 gms. $CuSO_4 \cdot 5H_2O$ are dissolved in 100 c.c. water and diluted to 150 c.c. This solution is added with constant stirring to the citrate carbonate solution. The mixture is immediately ready for use.

Bial's pentose reagent.
1 gram orcinol, dissolved in 500 c.c. 30% HCl, to which 30 drops of 10% ferric chloride have been added.

Bromine water. 25 c.c. of bromine in 1 liter of water.

Fehling's solution. Equal volumes of *A* and *B*.
A. 69.28 gms. of copper sulphate dissolved in water and made up to 1 liter.
B. 346 gms. of Rochelle salt (NaK tartrate), and 130 gms. of NaOH dissolved in water and made up to 1 liter.

Formalin. Commerical 40% solution of formaldehyde.

Gies' biuret reagent. This reagent consists of 2/1 normal NaOH solution to which enough 1/10 normal copper sulphate has been added to impart a slight though distinct blue color to the clear liquid. The copper sulphate should be added, drop by drop, with constant stirring.

Glyoxylic acid solution. Benedict's modification of Hopkins-Cole Reagent. Ten gms. of powdered magnesium are mixed with enough water to liberally cover the magnesium. 250 c.c. of saturated oxalic acid is now added slowly, and the solution kept cool, by allowing running water to flow over the flask during the addition of the acid. The magnesium oxalate is filtered off. The filtrate is acidified with acetic acid to prevent the partial precipitation of magnesium on long standing, and made up to 1 liter with water.

Magnesia mixture.

55 gms. magnesium chloride
70 gms. ammonium chloride
125 c.c. of NH_4OH (sp. gr. .880)
are dissolved in water and made up to 1 liter.

Magnesium sulphate (saturated solution).

600 gms. of cryst. $MgSO_4$ are dissolved in water and made up to 1 liter.

Millon's reagent.

400 gms. mercury (30 c.c.) are dissolved in 570 c.c. of conc. nitric acid. The solution is then diluted with two volumes of water.

Mörner's reagent.

Thoroughly mix 1 volume of formalin, 45 volumes of distilled water, and 55 volumes of conc. H_2SO_4.

Molisch's reagent.

144 gms. of alpha-naphthol are dissolved in alcohol and made up to 1 liter with alcohol.

Nylander's solution.

40 gms. Rochelle salt
20 gms. bismuth subnitrate
are dissolved in 1 liter of 2/1 NaOH.

Obermayer's reagent.

3 gms. of ferric chloride are dissolved in 1 liter of conc. hydrochloric acid.

Oxalic acid (saturated solution).

100 gms. of oxalic acid are dissolved in 1 liter of water.

Pavy's solution.

120 c.c. Fehling's solution
300 c.c. NH_4OH (sp. gr. .880)
are made up to 1 liter with water.

Phenol solution.

20 gms. phenol are diluted to 1 liter with water.

Phosphotungstic acid solution.

50 gms. of phosphotungstic acid and 30 c.c. of conc. H_2SO_4 are dissolved in water and made up to 1 liter.

Picric acid (saturated solution).

12 gms. of picric acid are dissolved in water and made up to 1 liter.

Sodium bisulphite (saturated solution).

600 gms. of sodium bisulphite are dissolved in water and made up to 1 liter.

Sodium chloride (saturated solution). 370 gms. of NaCl are dissolved in water and made up to 1 liter.

Schweitzer's reagent.

10 gms. of ammonium chloride are dissolved in 250 c.c. of 1/2 normal copper sulphate, and NaOH solution is added until precipitation is complete. The mixture is filtered and the pptd. cupric hydroxide is washed with water and then dissolved in 1 liter of conc. ammonium hydroxide.

Tannic acid solution.

100 gms. tannic acid
25 gms. sodium acetate
25 gms. sodium chloride
50 gms. glacial acetic acid
} are dissolved in water and made up to 1 liter.

Tartaric acid (saturated solution).

750 gms. of tartaric acid are dissolved in water and made up to 1 liter.

Trichloracetic acid solution.

100 gms. of trichloracetic acid are dissolved in water and made up to 1 liter.

Uffelmann's reagent.

Add 1/2 normal solution of ferric chloride to a 2% aqueous solution of phenol until an amethyst-blue is obtained.

INDICATORS

Alizarin red.

10 gms. sodium alizarin sulphonate are dissolved in water and made up to 1 liter.

Cochineal tincture.

5 gms. cochineal are extracted with 150 c.c. alcohol+100 c.c. water for several days; the solution is then filtered.

Congo red.

1 gm. Congo red is dissolved in water and made up to 1 liter.

Litmus.

10 gms. litmus are finely powdered and extracted with 50 c.c. of hot water. The blue liquid is decanted and made up to 1 liter.

Methyl orange.

1 gram methyl orange is dissolved in 500 c.c. alcohol and made up to 1 liter with water.

Methyl violet.

1 gram methyl violet is dissolved in water and made up to 1 liter.

Phenolphthalein.

10 gms. phenolphthalein are dissolved in alcohol and made up to 1 liter with alcohol.

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